JOURNAL OF AGRICULTURAL RESEARCH

VOL. 64 WASHINGTON, D. C., JANUARY 1, 1942 No. 1

REDUCING SOUTHERN SCLEROTIUM ROT OF SUGAR BEETS WITH NITROGENOUS FERTILIZERS [1]

By L. D. LEACH, *associate plant pathologist*, and A. E. DAVEY, *junior pomologist*, *California Agricultural Experiment Station* [2]

INTRODUCTION AND LITERATURE REVIEW

Several workers have advocated the use of soil amendments for controlling plant diseases caused by soil fungi. King and Loomis (*8*) [3] reported that heavy applications of manure or other organic material consistently reduced the number of cotton plants dying from phymatotrichum root rot. According to later work by King, Hope, and Eaton (*9*) and King (*7*), the benefits of organic manures resulted from inability of the root rot fungus to thrive in the presence of great numbers of bacteria, fungi, and other saprophytic organisms that are actively engaged in decomposing organic material. Neal and his coworkers (*15*), on the other hand, in 1933 demonstrated the toxicity of ammonia and certain ammonium salts to mycelium of *Phymatotrichum omnivorum* (Shear) Duggar and suggested that the effect of manure on the root rot may depend upon the early or ammoniacal decomposition of the manure. The following year Streets (*18*) reported experiments in which phymatotrichum root rot on deciduous fruit and nut trees was controlled with anhydrous ammonia or ammonium sulfate. In a later publication (*19*) he recommended either ammonium sulfate or ammonium phosphate for this disease on a number of perennial crops. The beneficial results were attributed primarily to the killing of the root rot fungus by the strong ammonium sulfate solution, secondarily to the stimulation of the plants by the large amount of quickly available nitrogenous fertilizers. Jordan and others (*6*) furnished further evidence of the effect of fertilizer treatments in reducing the loss of cotton from this fungus.

Applications of chicken manure, horse manure, or any of several other organic materials reduced the severity of take-all of wheat caused by *Ophiobolus graminis* Sacc. in the experiments of Fellows (*4*), who suggested the antagonistic effect of the natural soil microflora as the mechanism responsible for reducing the disease.

Walker and Musbach (*21*), after reviewing the results of earlier workers, presented additional evidence that root rot of canning peas caused by *Aphanomyces euteiches* Drechsler can be appreciably reduced by applications of mineral nitrogenous fertilizers. They suggest that the plants develop greater resistance to infection when supplied with quickly available nutrients. In a recent paper Smith and Walker (*16*) reported that reduction in aphanomyces root rot of peas was correlated with increase in total salt concentration of the nutrient

[1] Received for publication January 16, 1941. The field experiments reported in this paper were made possible through financial contributions by the beet-sugar companies operating in California and by the cooperation of numerous growers in the Sacramento Valley.

[2] The writers wish to thank Dr. M. W. Gardner and Dr. J. B. Kendrick for advice during the course of the investigations and the preparation of the manuscript.

[3] Italic numbers in parentheses refer to Literature Cited, p. 17.

solution and was not the specific effect of nitrogen, phosphorus, or potassium salts.

Club root of cabbage has been partly controlled with nitrogenous fertilizers. Under certain conditions, according to Walker and Larson (*20*), field applications of 400 to 800 pounds of $CaCN_2$ were fairly effective; and this material appeared about twice as effective, pound for pound, as $Ca(OH)_2$.

Early results with *Sclerotium rolfsii* Sacc. were not encouraging for this line of attack. Edgerton and Tims (*3*), reporting trials with 26 fertilizer combinations during two seasons in Louisiana, found no clear-cut differences between the various treatments. Molz (*14*), on the other hand, working in the island of San Miguel (Azores) with a sugar-beet root rot which he attributed to *Typhula betae* Rostr., recommended suitable fertilizers because the disease appeared less severe in fields abundantly provided with artificial fertilizers.

Although the writers have had no opportunity to examine cultures of *Typhula betae*, Dr. E. W. Schmidt, Zuckerfabrik Klein-Wanzleben, Magdeburg, Germany, has kindly furnished two cultures of *Typhula variabilis*, about which he made the following comment: "I take the liberty of sending you * * * a culture of a fungus * * * known in Europe under the name *Typhula variabilis*, which is very rare in this country and which appears only in dry-warm climates, as for example now and then in Italy and in Spain. I have found this fungus on sugar beet in South Spain near Sevilla. The symptoms of disease are exactly the same as those of *Sclerotium rolfsii*. Also in cultural respects, the fungus behaves in the same way, so that it is to be presumed that *Typhula variabilis* and *Sclerotium rolfsii* are possibly identical.[4]"

Examination of these cultures and observations on their growth on sugar beets have convinced the writers that this fungus is indistinguishable from *Sclerotium rolfsii*.

Encouraged by the report of Neal, Wester, and Gunn (*15*) of the toxicity of ammonia to *Phymatotrichum omnivorum* and by the partial control of the root rot caused by that fungus by means of either organic or inorganic nitrogenous fertilizers (*7*, *9*, *18*, *19*), the writers have conducted laboratory and field experiments to determine the possible application of similar methods for controlling *Sclerotium rolfsii* on sugar beets. The most striking feature of the results (*11*, *12*) has been the consistent reduction of infection in all trials where nitrogenous fertilizers have been heavily applied. The observed results might be explained by any of the following hypotheses: (1) Direct toxicity of the chemicals or their decomposition products to the sclerotia or mycelium of *S. rolfsii;* (2) creation of a soil medium less favorable to the growth or parasitism of the casual fungus; (3) alteration in the physiology or anatomy of the host as the result of nitrogen absorption that might decrease the susceptibility of the host; (4) alteration in the balance of micro-organisms in the soil resulting in the suppression of *S. rolfsii* because of antagonism or competition.

The field and laboratory experiments described in the following pages show the efficacy of nitrogenous fertilizers in reducing losses of sugar beets from southern sclerotium rot and contribute some evidence toward the evaluation of these hypotheses.

[4] Letter to Dr. J. B. Kendrick, dated February 19, 1935.

FIELD EXPERIMENTS ON THE EFFECT OF NITROGENOUS FERTILIZERS ON THE INCIDENCE OF *SCLEROTIUM ROLFSII* INFECTION ON SUGAR BEETS

TRIALS ON SOILS OF LOW FERTILITY

In a field of sugar beets (*Beta vulgaris L.*) in Yolo County, Calif., with a uniform infestation of *Sclerotium rolfsii*, a series of plots was established in the spring of 1934 to determine how heavy applications of anhydrous ammonia or ammonium sulfate might affect the subsequent development of the disease. Ammonia, dissolved in the irrigation water, was applied to six ⅛-acre plots at the rate of 142.5 pounds per acre (about 340 parts per million of irrigation water) on June 1, and at 103.5 pounds per acre (about 240 parts per million)

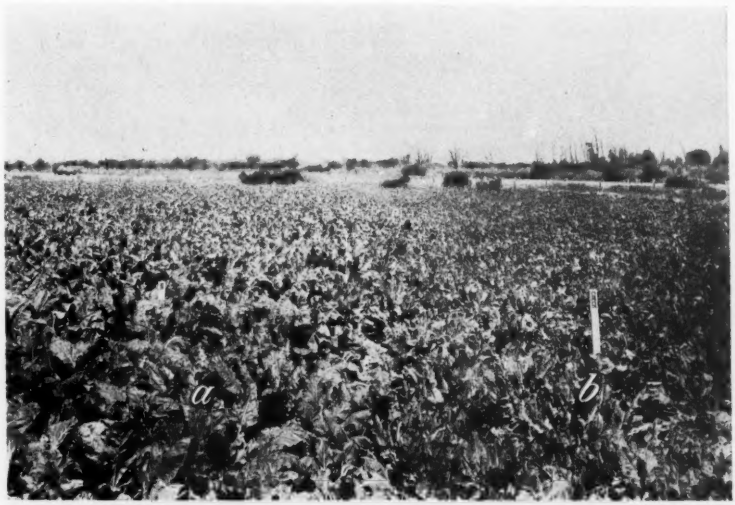


FIGURE 1.—Effect of nitrogenous fertilizers on sugar beets in field of low fertility but heavily infested with *Sclerotium rolfsii: a*, Anhydrous ammonia 246 pounds per acre, infection 13.5 percent, yield 17.1 tons per acre; *b*, nonfertilized, infection 34.0 percent, yield 10.3 tons per acre. For the average results from all plots see table 1. Similar results were obtained from ammonium sulfate applications.

on June 15, 1934. In the same way ammonium sulfate was dissolved in the irrigation water at the rate of 427.5 pounds per acre on each of these dates and applied to six ⅛-acre plots. An equal number of plots randomized among the fertilized plots were irrigated with water alone.

Soon after the ammonia or ammonium sulfate was applied to the plots the fertilized plants showed remarkable stimulation, as was apparent from the height of foliage, the size of leaves (fig. 1), and a change to a darker green in the color of the leaf. These responses undoubtedly indicate that there had been a deficiency of available nitrogen.

Counts of visibly infected beets in the middle four rows of each plot were made at 10-day intervals, starting before the first application was made and continuing until harvest on September 11. Mor-

tality curves for the fertilized and nonfertilized areas are presented in figure 2. On May 31, just before the first application, the average percentage of infection in the three groups of plots was approximately the same; but thereafter the mortality rate was much higher in nonfertilized plots than in those treated with either ammonia or ammonium sulfate.

All the plots were harvested on September 11 and 12, when final disease counts, yields, average root weights, and sucrose percentages were determined. Table 1 summarizes the results.

FIGURE 2.—Seasonal increase of *Sclerotium rolfsii* infection on sugar beets in fertilized and nonfertilized plots; average of six plots. The percentage of infection indicated by the circles over the date of September 12 includes the infected roots observed during harvest.

Infection was reduced from 30.0 percent in the nontreated plots to 7.6 and 9.2 percent in the plots treated with ammonia and ammonium sulfate. The nitrogenous fertilizers increased the average root weight by approximately 27 percent and depressed the sucrose percentage from 19.97 to 17.87 and 18.63 percent for ammonia and ammonium sulfate, respectively. The yield of sugar beets was increased from 11.5 tons to 19.9 and 19.6 tons per acre; the yield of gross sugar from 4,590 to 7,080 and 7,310 pounds per acre. To sum up, the percentage of disease-free plants, the average root weight, the yield of beets, and the gross sugar yield were significantly increased, while the sucrose percentage was significantly decreased, by both ammonia and ammonium sulfate.

TABLE 1.—*Comparative effects of anhydrous ammonia and ammonium sulfate applied in the irrigation water on the incidence of infection and the yield of sugar beets growing in soil of low fertility infested with Sclerotium rolfsii, 1934*

Treatment	Fertilizer applied per acre	Nitrogen applied per acre	Infected beets	Average root weight	Sucrose content of beets	Acre yield of—	
						Beets	Gross sugar [1]
	Pounds	*Pounds*	*Percent*	*Pounds*	*Percent*	*Tons*	*Pounds*
Control	None	None	30.0	1.20	19.97	11.5	4,590
Ammonia	[2] 246	200	7.6	1.51	17.87	19.9	7,080
Ammonium sulphate	[3] 855	176	9.2	1.53	18.63	19.6	7,310
Significant difference	(Odds 19:1		5.69	.20	.81	2.60	805
	(Odds 99:1		8.10	.28	1.16	3.70	1,145
Calculated *F* value [4]			48.00**	8.54**	17.88**	33.21**	34.76**

[1] Yield×sucrose percentage.
[2] Dissolved in irrigation water. First application, June 1, 142.5 pounds NH_3 per acre; second application, June 15, 103.5 pounds NH_3 per acre.
[3] Dissolved in irrigation water at the rate of 427.5 pounds of ammonium sulphate per acre in each of 2 applications, June 1 and 15, 1934.
[4] **Exceeds the 1-percent point, $F=7.56$ (17).

Evidently the increase in yield of sugar in the plots treated with ammonia or ammonium sulfate resulted in part from reduction of infection and in part from stimulation of growth. The slight reduction in sucrose percentage in the fertilized plots was more than offset by the increase in root size and the number of surviving beets.

Inasmuch as *Sclerotium rolfsii* caused considerable loss in the plots treated in 1934 before the treatments were applied, it was thought that the nitrogenous fertilizers should be applied early in the season for the most effective results. The 1935 trials were made to determine whether broadcasting the dry forms of nitrogenous fertilizers would be an effective means of application and to compare the relative efficiency of three forms of nitrogenous fertilizers. The field in Sutter County selected for the trials was last planted to sugar beets in 1931, when a loss of about 25 percent from southern sclerotium rot occurred.

On December 24, 1934, applications of 100 or 200 pounds of nitrogen as ammonium sulfate, calcium nitrate, and Cyanamid were each distributed over the surface of five $\frac{1}{12}$-acre plots, while an equal number of randomized plots were left untreated. The nitrogenous fertilizers were mixed with the soil by disking and harrowing, and the intention was to plant sugar beets after the lapse of 15 or 20 days. Because of heavy rainfall, however, planting was delayed until late in February; and then, because of unfavorable conditions, the stand was unsatisfactory. The field was replanted on April 1, and an excellent stand resulted. The beets grew satisfactorily until the second irrigation, when an excessive application of water to a poorly drained portion of the field resulted in the loss of numerous plants by "water rot" and necessitated the abandonment of one set (seven) of the experimental plots.

The remaining plots were harvested on September 4, 1935; the records appear in table 2. Although the percentage of infection is considerably less in the treated than in the nontreated plots, the differences between materials or rates of applications are not statistically significant. The explanation is, chiefly, that the number of replications was limited; that an area of severe infection occurred

in part of the two blocks; and that, despite chance randomization, some treatments were in a more favorable position than others.

TABLE 2.—*Comparative effects of various nitrogenous fertilizers, applied to the soil before planting, on the incidence of infection and the yield of sugar beets growing in soil of low fertility infested with Sclerotium rolfsii, 1935 and 1936*

Treatment	Fertilizer applied per acre	Nitrogen applied per acre	Infected beets	Average root weight	Sucrose content of beets	Acre-yield of—	
						Beets	Gross sugar [2]
1935 [1]							
	Pounds	*Pounds*	*Percent*	*Pounds*	*Percent*	*Tons*	*Pounds*
Control	None	None	15.92	1.03	19.25	12.23	4,700
Ammonium sulfate	485	100	7.82	1.19	19.17	16.53	6,280
Calcium nitrate	645	100	5.32	1.23	19.54	18.04	7,090
Cyanamid	475	100	8.35	1.16	19.18	15.86	6,080
Ammonium sulfate	970	200	6.45	1.37	19.02	18.36	6,990
Calcium nitrate	1,290	200	4.12	1.43	18.11	19.79	7,120
Cyanamid	950	200	6.87	1.28	18.08	17.69	6,360
Significant difference	Odds 19:1		7.31	.099	1.17	3.23	1,260
	Odds 99:1			.136	1.60	4.42	1,720
Calculated *F* value [3]			2.44	15.38**	2.14	5.00**	5.37**
1936 [4]							
Control	None	None	11.50	1.00	21.90	9.54	4,160
Cyanamid	238	50	9.43	1.15	21.10	12.07	5,110
Calcium nitrate	323	50	6.78	1.24	20.50	13.71	5,600
Cyanamid	475	100	6.00	1.20	20.80	13.72	5,680
Ammonium sulfate	485	100	7.36	1.20	20.30	13.90	5,650
Ammonium phosphate	623	100	9.60	1.26	20.80	13.16	5,440
Calcium nitrate	645	100	3.65	1.32	20.30	15.40	6,250
Cyanamid	950	200	2.87	1.45	18.90	16.58	6,250
Significant difference	Odds 19:1		4.64	0.19	1.39	1.84	489
	Odds 99:1		6.44	0.27	1.93	2.55	684
Calculated *F* value [5]			3.79*	4.25*	3.46*	12.11**	17.31**

[1] Date of planting, Apr. 1, 1935; date of application, Dec. 24, 1934; date of harvest, Sept. 4, 1935.
[2] Yield × sucrose pu.centage.
[3] *Exceeds the 5-percent point ($F=2.66$); ** exceeds the 1-percent point ($F=4.01$).
[4] Date of planting, Jan. 25, 1936; date of application, Dec. 18, 1935; date of harvest, Aug. 1–5, 1936.
[5] * Exceeds the 5-percent point ($F=2.77$); ** exceeds the 1-percent point ($F=4.28$).

Increasing the application from 100 pounds to 200 pounds of nitrogen decreased the percentage of infection only slightly. Conceivably the heavy rainfall (about 15 inches) between the date of application, December 24, and the final date of planting, April 1, leached much of the available nitrates from the root zone, thus tending to equalize the two rates of application. On the other hand, the residual effect of calcium nitrate was at least equal to that of the other compounds—a fact which suggests that leaching did not greatly affect the results, since this form of nitrogen is more readily leached than are ammonium forms of nitrogen. The tops of the fertilized beets responded strikingly to the added nitrogen; the increase in root size is shown by the data in table 2.

The tonnage of all treated plots was greatly increased over that of the nontreated controls, and at harvest time the heavier rates of application still showed a somewhat lower percentage of sucrose.

The applications increased the gross sugar yield between 1,400 and 2,400 pounds per acre. Although calcium nitrate produced a higher yield than the other materials, the differences may not be significant. An average of the three 100-pound nitrogen applications shows a gain

of 1,783 pounds of sugar per acre, or a 38 percent gain over nontreated plots. The 200-pound applications show an average gain of 2,123 pounds of sugar per acre, or a 45 percent increase over the controls.

During 1936 another opportunity was provided to test how various forms and amounts of nitrogenous fertilizers affect the incidence of southern sclerotium rot in a field of relatively low fertility.

A 17-acre block in a field infested with *Sclerotium rolfsii* in Sutter County was selected for the experiment. Nitrogen-bearing fertilizers were broadcast with a fertilizer distributor on December 18, 1935, and the soil was then disked and harrowed in preparation for a seedbed. Cyanamid was applied at the rate of 50, 100, and 200 pounds of nitrogen per acre; calcium nitrate at 50 and 100 pounds; ammonium sulfate and ammonium phosphate (16–20–0) at 100 pounds. The ammonium phosphate was included in order to learn whether phosphorus applied in addition to nitrogen would reduce the incidence of disease or increase the yield over that of nitrogen-treated plots. Each material was applied to three plots, 20 rows wide and 900 feet long (0.71 acre), and similar areas were left untreated. The seed was planted on January 25, and a good stand was obtained despite excessive rains that flooded part of the area and compacted the soil.

On March 12, a strong response of seedlings to the nitrogen fertilizers was exhibited in all plots except those receiving calcium nitrate and those in the nontreated area. After thinning, the calcium nitrate plots also showed a stimulation of leaf growth. The delay in response to this material can apparently be explained by the ease with which the nitrate is leached into the soil, though, as subsequent results indicate, little or no fertilizer material was leached beyond the ultimate root zone. All irrigation water was applied by sprinklers.

Diseased beets were counted in the four middle rows of each plot at intervals of 2 weeks from May 27 until harvest. All plots were harvested between August 1 and 5. To eliminate border effects, four rows were discarded between adjacent plots. When comparisons are made with the nontreated plots the incidence of disease is found to have been significantly reduced by each treatment except the lowest rate of Cyanamid, the ammonium sulfate, and the ammonium phosphate (table 2). Fifty pounds of nitrogen as calcium nitrate or 100 pounds as Cyanamid reduced the disease between 40 and 50 percent, whereas 100 pounds of nitrogen as calcium nitrate produced about 70 percent control, and 200 pounds as Cyanamid 75 percent control.

All treatments increased the average root weight; and although the percentages of sucrose were lower on the date of harvest, the tonnages from the fertilized plots were increased enough to provide an additional 1,000 to 2,100 pounds of gross sugar per acre.

As in the 1935 trials in an adjacent district, nitrogen from calcium nitrate produced a higher yield and held the disease at a lower level than the same amount of nitrogen from the other materials. Whether calcium nitrate is especially favorable for use on the heavier soils of northern Sutter County can be determined only by future trials. Its effect on the incidence of disease is especially significant because calcium nitrate is nontoxic to sclerotia or mycelium of *Sclerotium rolfsii*, as will be shown later in this report.

Since ammonium phosphate neither reduced the incidence of disease nor increased the yield as compared with the same amount of nitrogen from other sources, it may be concluded that at least under

the conditions of this experiment the addition of phosphorus was not beneficial.

The three foregoing experiments are typical of five made on soils of low fertility in that the percentage of infection was reduced by heavy applications of nitrogenous fertilizers, the yield of beets was strikingly increased, and the combined benefits from suppressing the incidence of disease and from crop stimulation considerably exceeded the cost of the treatments.

During the same years eight additional trials were conducted in fields which, judging by the plant growth, were abundantly supplied with available food elements. Detailed data from three such experiments follow.

TRIALS ON SOILS OF HIGH FERTILITY

In the hope of finding a soil amendment that could be applied to the soil to make possible the profitable production of sugar beets on land infested with *Sclerotium rolfsii*, four materials were applied. Manure at 10 tons per acre, hydrated lime at 5 tons per acre, and Cyanamid at 1,000 pounds per acre were incorporated in the soil on February 1. Ammonium sulfate was applied as a side dressing to the growing plants at 1,000 pounds per acre on May 16.

Five replications of each treatment and of nontreated plots, one eighty-second of an acre in size, were randomized in a linear series. Irrigation water was passed through 40-mesh screens in metal dams to prevent the introduction of sclerotia to treated plots. Throughout the growing season, the visibly infected plants in the middle four rows of each plot were counted at 10-day intervals. Table 3 presents data on the incidence of infection and yield from each treatment.

TABLE 3.—*Comparative effects of various soil amendments applied before planting on the incidence of infection and the yield of sugar beets growing in fertile soil infested with Sclerotium rolfsii, 1934* [1]

Treatment	Amount applied per acre	Infected beets	Average root weight	Sucrose content of beets	Acre yield of—	
					Beets	Gross sugar
	Pounds	*Percent*	*Pounds*	*Percent*	*Tons*	*Pounds*
Control	None	59.3	1.88	17.72	7.81	2,780
Hydrated lime [2]	10,000	45.5	1.80	17.40	10.92	3,820
Manure [2]	20,000	38.8	1.76	17.30	11.12	3,870
Ammonium sulfate [3]	1,000	50.1	2.00	15.32	10.76	3,270
Cyanamid [3]	1,000	24.8	1.86	15.56	16.83	5,220
Significant difference	{Odds 19:1	14.6	.29	.67	2.85	960
	{Odds 99:1	20.1	.40	.92	3.92	1,320
Calculated *F* value [4]		7.03**	.89	25.37**	11.95**	8.22**

[1] Date of planting: Mar. 8, 1934.
[2] Applied before planting, Feb. 1.
[3] Applied as a side dressing, May 16.
[4] *Exceeds the 5-percent point ($F=3.01$); **exceeds the 1-percent point ($F=4.77$).

The number of infected plants was considerably smaller in the Cyanamid-treated plots than in any of the others. Although manure and hydrated lime also proved somewhat beneficial, the side-dressing of ammonium sulfate apparently did not reduce the percentage of infection significantly below that of the nontreated plots.

Both the Cyanamid-treated and the ammonium-sulfate-treated plots showed a decided increase in green color and size of foliage be-

cause of the heavy nitrogen application, but the average root weight was not significantly increased over that of other plots. The sucrose percentage at harvest (August 11) showed a depression of approximately 2 percent in the ammonium sulfate and Cyanamid plots.

In total yield of sugar beets, the Cyanamid-treated plots showed a gain of 9 tons per acre over the nontreated plots and about 6 tons per acre over the other treatments. This gain was due almost entirely to the prevention of infection, since the differences in average root weight were not significant. In gross sugar yield there was a gain of 2,400 pounds in the Cyanamid-treated over the nontreated plots and a gain of approximately 1,400 pounds over the lime- and manure-treated plots. The same amount of nitrogen applied as a side dressing of ammonium sulfate failed to produce a significant improvement in survival of plants or in yield over the nontreated plots.

Under the conditions of this experiment, Cyanamid at the rate of 1,000 pounds per acre considerably reduced the percentage of infection and made possible the production of a profitable crop, whereas the other materials used (manure, lime, and ammonium sulfate as a side dressing) did not produce sufficient improvement to justify their use.

The most striking features of the results are (1) the suppression of infection by heavy application of Cyanamid even though no increase in root size occurred, and (2) the failure of equal amounts of ammonium sulfate to produce similar effects when sidedressed at a shallow depth comparatively late in the growing season. Most likely the ammonium sulfate was leached into the soil too late to produce the effects obtained by applying the Cyanamid before planting. Supplementary observations and investigations apparently preclude the possibility of a specific toxic effect of Cyanamid on *Sclerotium rolfsii* in field applications.

One experiment during 1935 was conducted on a very fertile plot of land adjoining the Sacramento River on Merritt Island in Yolo County. Comparisons were made of applications of 50 to 200 pounds of nitrogen per acre as anhydrous ammonia, ammonium sulfate, or calcium nitrate dissolved in the surface irrigation water. Eight treatments were each replicated six times on 1/20-acre plots in randomized blocks. Because of the high water level in the Sacramento River, the soil in part of the experimental area was saturated, and some water flowed across the surface of the soil from seepage under the protecting levee during March and April. As a result, only two of the six blocks provided conditions favorable for the experiment.

Since the data (table 4) consist of averages from only these two blocks, no attempt has been made to calculate the significance of differences.

Judging from the results, the reduction of infection from *Sclerotium rolfsii* was in proportion to the amount of nitrogen applied, and the form of nitrogen exerted little influence. It should be noted that in this field there was no evidence that the fertilizers increased the size of the roots. All increases either in tonnage or in gross sugar were apparently due to the reduction of infection by *S. rolfsii*. The large average root weights of the nontreated beets may have been due to the fact that large numbers of plants were killed by the fungus early in the growing season and that the survivors were relatively free from competition with adjacent plants; or possibly the smaller beets in the nontreated

plots were infected at an early date, thus permitting the survival of only the larger plants.

TABLE 4.—*Comparative effects of various nitrogenous fertilizers applied in the irrigation water on the incidence of infection and the yield of sugar beets growing in fertile soil infested with Sclerotium rolfsii, 1935*

Treatment	Fertilizer applied per acre	Nitrogen applied per acre	Infected beets	Average root weight	Sucrose content of beet	Acre yield of—	
						Beets	Gross sugar [1]
	Pounds	*Pounds*	*Percent*	*Pounds*	*Percent*	*Tons*	*Pounds*
Control	None	None	72.7	2.12	17.3	6.26	2,170
Ammonia	62	50	60.0	1.80	16.8	8.42	2,840
Ammonium sulfate	245	50	53.7	1.75	16.9	9.80	3,285
Ammonia	124	100	43.8	1.67	17.4	12.66	4,360
Ammonium sulfate	490	100	42.3	1.84	16.3	13.15	4,265
Ammonia	248	200	23.0	1.66	15.7	18.05	5,670
Ammonium sulfate	980	200	30.5	1.69	17.1	14.69	5,000
Calcium nitrate	1,290	200	33.4	1.72	16.7	13.94	4,640

[1] Yield×sucrose percentage.

During 1937 two series of plots were established in a field near Knights Landing, Sutter County, to show the effect of moderate applications of ammonium sulfate or anhydrous ammonia on the incidence of southern sclerotium rot in a relatively fertile field. An area was selected which, according to soil samples, showed a population of about 65 sclerotia per square foot, sufficient to produce an infection of 5 to 8 percent (*13*) by August 1.

In one part of the field, ammonium sulfate was applied at the rate of 100 pounds of nitrogen per acre to six 16-row plots of sugar beets alternating with nonfertilized strips of the same width. One-half of the material was applied with the seed on April 5; the other half was side dressed on June 4, just before the first irrigation. Since very little rain fell between these dates, presumably little or none of the fertilizer was in solution and available to the plants until after the irrigation.

In another part of the same field, a similar series of plots was arranged in cooperation with the Association Laboratories of Anaheim, Calif. In this series, five 16-row plots were fertilized with anhydrous ammonia applied in the first irrigation at the rate of 100 pounds of nitrogen per acre, and an equal number of adjacent plots were left unfertilized.

Before the first irrigation, and at intervals of 2 weeks thereafter, the number of infected beets in each plot was determined. The rate of increase in the fertilized and nonfertilized plots is shown in figure 3, *A* and *B*. These infection curves show that both ammonium sulfate and ammonia strikingly reduced the percentage of infection. This effect was noticeable in the ammonia-treated plots within 2 weeks after the application, but a longer period was required in the ammonium sulfate plots. Since these two materials have in previous trials produced similar results when both were dissolved in the irrigation water, apparently the differences in this case were due mainly to the method of application, the side-dressed material being less quickly available to the plants.

In the same way the ammonia-treated plots showed a striking stimulation in top growth soon after the application, whereas the

ammonium-sulfate-treated plots gave a slower and less evident response. Table 5 shows the differences in the average percentage of infection and yield of the two series of plots.

The ammonium-sulfate-treated plots showed an average increase of 2.8 tons per acre and about 700 pounds of sugar per acre over the nontreated plots. The sucrose percentage appeared slightly lower in the fertilized plots, but the difference was not statistically sig-

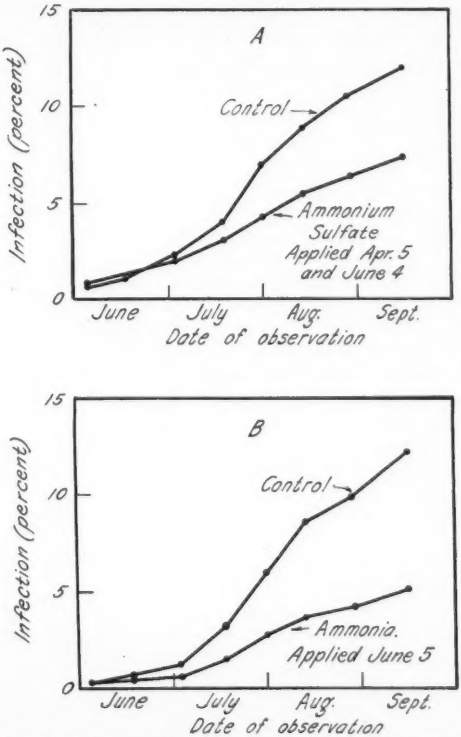


FIGURE 3.—Effect of nitrogenous fertilizers on infection of sugar beets by *Sclerotium rolfsii: A*, Ammonium sulfate side-dressed at time of planting and just before first irrigation in comparison with nonfertilized plots; *B*, anhydrous ammonia dissolved in first irrigation water. Both materials applied at the rate of 100 pounds of nitrogen per acre.

nificant. Because of the variation in the sucrose percentage of the plots, the increase in yield of sugar per acre of the fertilized over the nonfertilized plots appears not to be statistically significant, though the yield in tons per acre shows significant differences. The ammonia-treated plots showed an average increase of 4.7 tons of beets per acre and 1,200 pounds of sugar per acre over the nontreated plots. As in the other series, the apparent reduction in sucrose percentage was not significant. Since the two series of plots were located in different parts of the field, the increases in yield cannot be compared directly. Furthermore, the apparent superiority of the anhydrous ammonia is

plots were infected at an early date, thus permitting the survival of only the larger plants.

TABLE 4.—*Comparative effects of various nitrogenous fertilizers applied in the irrigation water on the incidence of infection and the yield of sugar beets growing in fertile soil infested with Sclerotium rolfsii, 1935*

Treatment	Fertilizer applied per acre	Nitrogen applied per acre	Infected beets	Average root weight	Sucrose content of beet	Acre yield of—	
						Beets	Gross sugar [1]
	Pounds	*Pounds*	*Percent*	*Pounds*	*Percent*	*Tons*	*Pounds*
Control	None	None	72.7	2.12	17.3	6.26	2,170
Ammonia	62	50	60.0	1.80	16.8	8.42	2,840
Ammonium sulfate	245	50	53.7	1.75	16.9	9.80	3,285
Ammonia	124	100	43.8	1.67	17.4	12.66	4,360
Ammonium sulfate	490	100	42.3	1.84	16.3	13.15	4,265
Ammonia	248	200	23.0	1.66	15.7	18.05	5,670
Ammonium sulfate	980	200	30.5	1.69	17.1	14.69	5,000
Calcium nitrate	1,290	200	33.4	1.72	16.7	13.94	4,640

[1] Yield×sucrose percentage.

During 1937 two series of plots were established in a field near Knights Landing, Sutter County, to show the effect of moderate applications of ammonium sulfate or anhydrous ammonia on the incidence of southern sclerotium rot in a relatively fertile field. An area was selected which, according to soil samples, showed a population of about 65 sclerotia per square foot, sufficient to produce an infection of 5 to 8 percent (*13*) by August 1.

In one part of the field, ammonium sulfate was applied at the rate of 100 pounds of nitrogen per acre to six 16-row plots of sugar beets alternating with nonfertilized strips of the same width. One-half of the material was applied with the seed on April 5; the other half was side dressed on June 4, just before the first irrigation. Since very little rain fell between these dates, presumably little or none of the fertilizer was in solution and available to the plants until after the irrigation.

In another part of the same field, a similar series of plots was arranged in cooperation with the Association Laboratories of Anaheim, Calif. In this series, five 16-row plots were fertilized with anhydrous ammonia applied in the first irrigation at the rate of 100 pounds of nitrogen per acre, and an equal number of adjacent plots were left unfertilized.

Before the first irrigation, and at intervals of 2 weeks thereafter, the number of infected beets in each plot was determined. The rate of increase in the fertilized and nonfertilized plots is shown in figure 3, *A* and *B*. These infection curves show that both ammonium sulfate and ammonia strikingly reduced the percentage of infection. This effect was noticeable in the ammonia-treated plots within 2 weeks after the application, but a longer period was required in the ammonium sulfate plots. Since these two materials have in previous trials produced similar results when both were dissolved in the irrigation water, apparently the differences in this case were due mainly to the method of application, the side-dressed material being less quickly available to the plants.

In the same way the ammonia-treated plots showed a striking stimulation in top growth soon after the application, whereas the

ammonium-sulfate-treated plots gave a slower and less evident response. Table 5 shows the differences in the average percentage of infection and yield of the two series of plots.

The ammonium-sulfate-treated plots showed an average increase of 2.8 tons per acre and about 700 pounds of sugar per acre over the nontreated plots. The sucrose percentage appeared slightly lower in the fertilized plots, but the difference was not statistically sig-

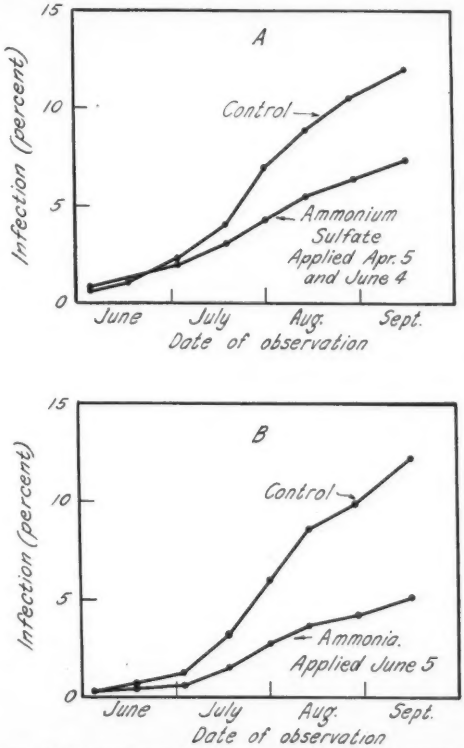


FIGURE 3.—Effect of nitrogenous fertilizers on infection of sugar beets by *Sclerotium rolfsii: A*, Ammonium sulfate side-dressed at time of planting and just before first irrigation in comparison with nonfertilized plots; *B*, anhydrous ammonia dissolved in first irrigation water. Both materials applied at the rate of 100 pounds of nitrogen per acre.

nificant. Because of the variation in the sucrose percentage of the plots, the increase in yield of sugar per acre of the fertilized over the nonfertilized plots appears not to be statistically significant, though the yield in tons per acre shows significant differences. The ammonia-treated plots showed an average increase of 4.7 tons of beets per acre and 1,200 pounds of sugar per acre over the nontreated plots. As in the other series, the apparent reduction in sucrose percentage was not significant. Since the two series of plots were located in different parts of the field, the increases in yield cannot be compared directly. Furthermore, the apparent superiority of the anhydrous ammonia is

probably due, at least in part, to the time and method of application, as previously mentioned.

TABLE 5.—*Comparative effects of ammonium sulfate applied to the soil and anhydrous ammonia applied in the irrigation water on the incidence of infection and the yield of sugar beets growing in soil infested with Sclerotium rolfsii, 1937*

Series and treatment	Nitrogen applied per acre	Infected beets	Sucrose content of beets	Acre yield of—	
				Beets	Gross sugar [1]
Series 1:	*Pounds*	*Percent*	*Percent*	*Tons*	*Pounds*
Control	None	11.97	15.36	18.48	5,692
Ammonium sulfate	100	7.30	15.09	21.28	6,397
Significant difference, 19:1		3.05	2.38	2.14	1,170
Series 2:					
Control	None	12.20	16.91	21.81	7,380
Ammonia	100	5.06	16.17	26.53	8,600
Significant difference, 19:1		5.00	1.11	3.77	1,010

[1] Yield×sucrose percentage.

DISCUSSION OF EXPERIMENTS ON NITROGEN FERTILIZERS IN RELATION TO SOUTHERN SCLEROTIUM ROT

During the 4 years from 1934 to 1937 inclusive, 13 separate replicated experiments were conducted in various parts of the Sacramento Valley to determine the relation of nitrogenous fertilizers to the incidence of southern sclerotium rot of sugar beets. Table 6 summarizes the results.

TABLE 6.—*Summary of 13 experiments on the relation of nitrogenous fertilizers to the incidence of Sclerotium rolfsii infection on sugar beets*

Treatment	Trials	Relative amount of infection [1]	
		Range	Mean
	Number	*Percent*	*Percent*
Control	13		100
50 pounds of nitrogen per acre as—			
Ammonia	1		82.5
Ammonium sulfate	2	61.9–73.9	67.9
Calcium nitrate	1		59.1
Cyanamid	1		81.1
Average for 50 pounds of nitrogen per acre			71.8
100 pounds of nitrogen per acre as—			
Ammonia	4	19.7–60.2	40.8
Ammonium sulfate	8	43.8–64.4	53.6
Calcium nitrate	6	21.4–61.0	36.2
Cyanamid	5	35.7–80.9	54.7
Average for 100 pounds of nitrogen per acre			46.3
200 pounds of nitrogen per acre as—			
Ammonia	3	25.3–31.6	28.0
Ammonium sulfate	6	9.5–[2] 84.5	41.1
Calcium nitrate	4	16.1–45.9	33.3
Cyanamid	5	25.0–51.9	39.0
Average for 200 pounds of nitrogen per acre			35.4

[1] The relative amount of infection in each experiment is equal to 100 times the ratio of the percentage of infection in the fertilized plots to that in the nonfertilized plots.

[2] Failure of control was apparently due to shallow side dressing of the ammonium sulfate late in the growing season.

There was considerable variability in the average percentage of infection in the nontreated plots of the different experiments. For direct comparison, the results must be converted to a relative basis. Accordingly, the average percentage of infection in the nontreated plots in each experiment has been considered as 100, while the percentage of infection in the fertilized plots in the same experiment is represented as a given percentage of that amount. For example, the average of all experiments in which 50 pounds of nitrogen was added shows 71.8 percent as much disease as in the nontreated plots of the same experiments. In the same way 100 pounds of nitrogen per acre showed an average of 46.3 percent as much disease as in the control plots; 200 pounds of nitrogen, 35.4 percent. The degree of control obtained by using the same material in different experiments usually showed greater differences than were obtained by the use of different forms of nitrogen in the same experiment. No convincing proof has, therefore, been obtained that one source of nitrogen surpasses another for use over the wide range of conditions covered by these experiments. In all cases, the greater the dosage of a given material, the greater the degree of control obtained, although occasionally the differences were not statistically significant.

In some experiments the fertilizers were broadcast in advance of planting; in others the materials were dissolved in the irrigation water; and in three cases the fertilizers were applied as a side dressing to the growing crop. Satisfactory results have been obtained with each of these methods of application, and the results show no consistent differences in favor of any certain time or method. In general, however, applications that promoted the most vigorous vegetative growth of the beets were most effective in retarding the infection by *Sclerotium rolfsii*. For satisfactory results, the applications must be made before any large percentage of beets has been infected.

Planting of heavily infested fields with sugar beets in the hope of controlling the disease with nitrogen fertilizers is to be discouraged; judging from the results obtained in the present experiments, no more than a 50 to 75 percent control can be expected. In lightly or moderately infested fields the percentage of infection has often been kept low, but the increase in yield due to disease control will seldom balance the cost of the material. Economical use of this method of control is limited, theretofore, to fields in which nitrogen fertilizers increase the yield of sugar per acre enough to equal at least the cost of the fertilizer and the other costs incidental to its usage.

TOXICITY OF NITROGEN COMPOUNDS TO *SCLEROTIUM ROLFSII*

LABORATORY STUDIES

In view of the striking reduction of infection on sugar beets produced by nitrogenous fertilizers, it is interesting to consider how these materials affect the causal fungus. As shown by laboratory studies previously reported (*1*, *2*, *12*), anhydrous ammonia in solution (ammonium hydroxide) is very toxic to sclerotia and mycelium of *Sclerotium rolfsii*, being of the same order of fungicidal power as formaldehyde.

Salts of ammonia such as ammonium sulfate and ammonium phosphate have also proved toxic to mycelium of the fungus, but only in alkaline solutions. In these trials, units of *Sclerotium rolfsii*

mycelium were obtained by germinating surface-sterilized sclerotia upon filter paper moistened with standard beef broth diluted to one-twenty-fifth strength with water. After the mycelium had been allowed to grow for 4 days at 30° C. the filter paper bearing the mycelium was cut into small squares with sides of 0.5 cm. Twenty of these squares, constituting a test sample, were immersed in the test solutions in shell vials for definite periods at room temperature. They were then washed for not less than 5 minutes in tap water and plated on moist nonsterilized peat soil that had been passed through a 40-mesh screen. It was found that solutions of ammonium sulfate with an ammonium content equivalent to 82.4 millimols of ammonia

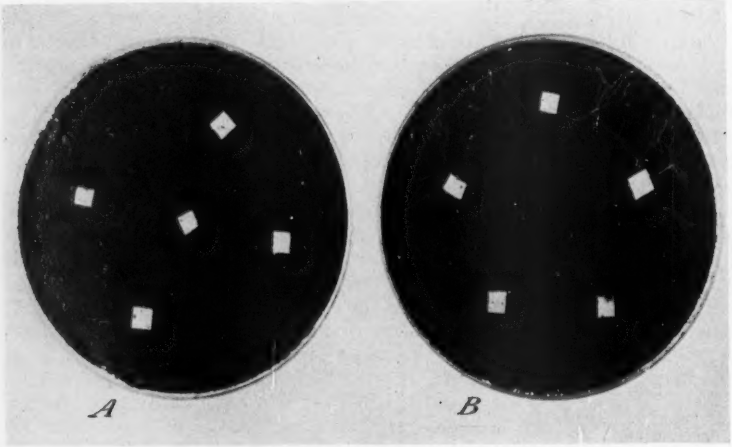


FIGURE 4.—Effect of calcium carbonate on the toxicity of ammonium sulfate to mycelium of *Sclerotium rolfsii* as indicated by: (*A*), Absence of growth after immersion in a solution containing 1,400 p. p. m. of ammonium sulfate in addition to an excess of calcium carbonate. (*B*) Growth of mycelium after immersion in a saturated solution of calcium carbonate at pH 8.4 for 24 hours. Similar growth followed immersion in a 1,400 p. p. m. solution of ammonium sulfate. The substratum was nonsterilized peat soil.

(NH_3) per liter (pH 6.0) and solutions of monammonium phosphate with the same ammonia content (pH 4.5) were nonlethal to mycelium of *Sclerotium rolfsii* immersed in them for 5 days. In the same way a saturated solution of calcium carbonate ($CaCO_3$) was nonlethal at pH 8.4 (quinhydrone electrode). The addition of calcium carbonate to the solution of ammonium sulfate containing the equivalent of 41.2 millimols of NH_3 and giving a pH of 7.8 resulted in the death of 96 to 100 percent of the mycelial units (fig. 4). Solutions of ammonium bicarbonate and dibasic ammonium phosphate, with a pH of 7.5 with equivalents of 17.8 and 14.7 millimols of ammonia respectively, were also lethal within 5 days to immersed mycelium. These results suggest that the toxicity of ammonium sulfate is due to the release of NH_3 by hydrolysis in an alkaline solution. In alkaline soils applications of anhydrous ammonia or ammonium sulfate may result in conditions similar to those in the solutions buffered with calcium carbonate, and thus it appeared possible that ammonia in

solution might be the effective agent in reducing the extent of infection by inhibiting fungus growth.

Calcium cyanamide in aqueous solution appeared to be only mildly toxic to this fungus. Mycelium was killed, however, after immersion for 120 hours in a solution containing 1,500 p. p. m. of dry commercial Cyanamid.

Calcium nitrate exhibited no fungicidal effect on mycelium or sclerotia of *Sclerotium rolfsii;* and in one trial there was some evidence that the fungus growth was stimulated by applications of this material.

The formation of NH_3 from calcium nitrate in toxic amounts by denitrification processes in well-aerated soils seems highly improbable in view of the experience of E. L. Proebsting (unpublished data), who has found that in orchard soils to which calcium nitrate was added the NH_3 content was not detectable by ordinary methods of analysis.

FIELD TRIALS

If, as has been suggested, the partial control of the southern sclerotium root rot is due to the toxicity of ammonium compounds or Cyanamid, then field trials should demonstrate an appreciable reduction in number or viability of sclerotia after such applications.

In one series of trials, diluted ammonium hydroxide was applied to the surface of duplicate plots of heavily infested soil at the rate of 3,000 pounds of ammonia per acre. Ten days later sclerotia recovered from the ammonia-treated areas averaged 90.5 percent germination, whereas those recovered from the nontreated areas averaged 86.7 percent germination.

In another series of plots Cyanamid was broadcast on the surface of heavily infested plots at the rate of 945 pounds (200 pounds of nitrogen) per acre and mixed with the soil by disking. Soil samples were collected from duplicate plots at intervals of 33, 60, and 115 days after treatment. Table 7 shows the number of sclerotia recovered from treated and nontreated areas, together with the average germination of the sclerotia.

TABLE 7.—*Germinability of sclerotia recovered from field soils after treatment with Cyanamid*

Period exposed in soil (days)	Rate of application of Cyanamid per acre	Sclerotia recovered per 800 gm. of soil	Average germination
	Pounds	*Number*	*Percent*
33	None	91	95
	945	109	80
60	None	67	87
	945	71	92
115	None	89	99
	945	73	91

Evidently, neither anhydrous ammonia nor Cyanamid applied to the surface of infested soils produced any appreciable toxic effects on the sclerotia under the conditions of these trials. These observations do not, however, preclude the possibility that the chemicals may have retarded germination of sclerotia, inhibited growth of mycelium, reduced its infective ability, or even destroyed the mycelium of the causal fungus within the soil.

DISCUSSION

Evidence has been presented to show that applications of anhydrous ammonia, ammonium sulfate, Cyanamid, or calcium nitrate will appreciably reduce the amount of southern sclerotium root rot in sugar beets. In solution the first three materials exhibit various degrees of toxicity to the mycelium of *Sclerotium rolfsii*, whereas at similar concentrations calcium nitrate is nonlethal. In field soils excessive applications of anhydrous ammonia and Cyanamid have failed to kill sclerotia of this fungus.

Ammonium compounds in alkaline media liberate sufficient NH_3 to provide a low degree of toxicity to mycelium, and this phenomenon may explain their partial control of sclerotium root rot. The fact that calcium nitrate, which is itself nonlethal to mycelium and apparently does not liberate NH_3 in toxic amounts, is equally effective in reducing sclerotium rot suggests that this reduction is probably not due to the toxicity of nitrogen compounds to the causal fungus.

While there is evidence against the acceptance of ammonia as a toxic agent in these field trials, there is information from investigations by Higgins (*5*) and Krüger (*10*) which suggests that the presence of ammonia or other basic substances may so affect the metabolic activity of the fungus as to reduce its ability to injure or invade the tissue of the sugar beet. Both Higgins and Krüger agree concerning the importance of oxalic acid produced by the fungus in the processes of infection. Higgins has also shown that substrates of high nitrogen content result in the formation of considerable amounts of ammonium oxalate which he found to be much less toxic than oxalic acid.

A second possible explanation for the reduced infection is that it may be due to a physiological or anatomical resistance induced in the plants. There appeared to be a close correlation between nitrogen stimulation to sugar beets (as exhibited by leaf and root growth) and the retardation in the development of the disease. The effect of nitrogenous fertilizers in reducing the disease was nearly as pronounced in fields of high fertility as in fields of low fertility, which would suggest that any resistance contributed to the sugar beet is associated with a luxury consumption of nitrogen on the part of the host rather than with the correction of a deficiency as usually understood.

The possibility remains that the application of large amounts of nitrogen compounds may have stimulated certain micro-organisms to pronounced multiplication and activity and in some way this biologic change suppressed *Sclerotium rolfsii*, thus preventing the usual amount of infection.

Neither the writers' investigations nor those of others provide an adequate basis for evaluating the relative importance of the above hypotheses.

SUMMARY

Applications of nitrogenous fertilizers in field plots have consistently reduced the percentage of *Sclerotium rolfsii* infection on sugar beets. Ammonium sulfate, anhydrous ammonia, calcium nitrate, and Cyanamid proved equally effective when equivalent amounts of nitrogen were supplied under favorable conditions. According to the averages of all trials, 50 pounds of nitrogen per acre reduced infection

by about 28 percent, 100 pounds by 54 percent, and 200 pounds by 65 percent, as compared with nonfertilized areas. Yields were increased by these treatments both because of the greater number of disease-free beets and because of the stimulation of growth produced by the fertilizers in most of the fields. Economical use of this method of control is limited to areas or fields where sugar beets respond to nitrogenous fertilizers.

According to laboratory trials, low concentrations of ammonia in aqueous solution are toxic to mycelium and sclerotia of *Sclerotium rolfsii*. Ammonium sulfate in alkaline solutions is also mildly toxic to mycelium. Calcium nitrate in solutions of similar concentrations was nontoxic to mycelium or sclerotia.

The fact that these three materials, differing in toxic relations, were equally effective in preventing infection from *Sclerotium rolfsii* suggests that the control obtained may have been due to factors other than ammonium toxicity.

Heavy applications of anhydrous ammonia and Cyanamid to naturally infested soils failed to destroy the sclerotia or to reduce their viability. The effects upon mycelium in the soil were not determined.

To explain the partial control of southern sclerotium rot on sugar beets by nitrogenous fertilizers, it is suggested that changes in the metabolism of the causal fungus may reduce its growth or pathogenicity; that resistance of the host may be increased by alteration of its metabolism and anatomy; or that there may be a suppression of the causal organism because of a change in the balance of micro-organisms in the soil.

LITERATURE CITED

(1) Davey, A. E., and Leach, L. D.
1935. toxicity of compounds of ammonia to sclerotium rolfsii. (Abstract) Phytopathology 25: 895–896.
(2) ——— and Leach, L. D.
1941. experiments with fungicides for use against sclerotium rolfsii in soils. Hilgardia 13: 523–547.
(3) Edgerton, C. W., and Tims, E. C.
1936. diseases of sugar beets in louisiana. La. Agr. Exp. Sta. Bul. 273, 12 pp., illus.
(4) Fellows, Hurley.
1929. studies of certain soil phases of the wheat take-all problem. (Abstract) Phytopathology 19: 103.
(5) Higgins, B. B.
1927. physiology and parasitism of sclerotium rolfsii sacc. Phytopathology 17: 417–448, illus.
(6) Jordan, H. V., Dawson, P. R., Skinner, J. J., and Hunter, J. H.
1934. the relation of fertilizers to the control of cotton root rot in texas. U. S. Dept. Agr. Tech. Bul. 426, 76 pp., illus.
(7) King, C. J.
1937. a method for the control of cotton root rot in the irrigated southwest. U. S. Dept. Agr. Cir. 425, 10 pp., illus.
(8) ——— and Loomis, H. F.
1926. experiments on the control of cotton root rot in arizona. Jour. Agr. Res. 32: 297–310, illus.
(9) ——— Hope, Claude, and Eaton, E. D.
1934. some microbiological activities affected in manurial control of cotton root rot. Jour. Agr. Res. 49: 1093–1107, illus.
(10) Krüger, Heinrich.
1939. sclerotium rolfsii sacc. an der zuckerrübe. Kühn-Arch. 48: [233]–281, illus.

432645—41——2

(11) LEACH, L. D., and DAVEY, A. E.
1935. SOIL AMENDMENTS FOR SOUTHERN SCLEROTIUM ROT OF SUGAR BEETS. (Abstract) Phytopathology 25: 896.
(12) ——— and DAVEY, A. E.
1935. TOXICITY OF LOW CONCENTRATIONS OF AMMONIA TO MYCELIUM AND SCLEROTIA OF SCLEROTIUM ROLFSII. (Phytopath. Note). Phytopathology 25: 957–959; illus.
(13) ——— and DAVEY, A. E.
1938. DETERMINING THE SCLEROTIAL POPULATION OF SCLEROTIUM ROLFSII BY SOIL ANALYSIS AND PREDICTING LOSSES OF SUGAR BEETS ON THE BASIS OF THESE ANALYSES. Jour. Agr. Res. 56: 619–631, illus.
(14) MOLZ, E.
1920. DIE TYPHULA–FÄULE DER ZUCKERRÜBEN AUF DEN AZOREN UND IHRE BEKÄMPFUNG. Ztschr. f. Pflanzenkrank. 30: [121]–139, illus.
(15) NEAL, DAVID C., WESTER, R. E., and GUNN, KENNETH C.
1933. GROWTH OF THE COTTON ROOT-ROOT FUNGUS IN SYNTHETIC MEDIA, AND THE TOXIC EFFECT OF AMMONIA ON THE FUNGUS. Jour. Agr. Res. 47: 107–118, illus.
(16) SMITH, PAUL G., and WALKER, J. C.
1941. CERTAIN ENVIRONAL AND NUTRITIONAL FACTORS AFFECTING APHANOMYCES ROOT ROT OF GARDEN PEA. Jour. Agr. Res. 63: 1–20, illus.
(17) SNEDECOR, GEORGE W.
1934. CALCULATION AND INTERPRETATION OF ANALYSIS OF VARIANCE AND COVARIANCE. 96 pp. Ames, Iowa. (Iowa State Col., Div. Indus. Sci. Monog. 1.)
(18) STREETS, R. B.
1934. THE TREATMENT OF DECIDUOUS FRUIT TREES AND NUT TREES INFECTED BY PHYMATOTRICHUM OMNIVORUM WITH AMMONIUM COMPOUNDS. Science (n. s.) 79: 417–418.
(19) ———
1937. PHYMATOTRICHUM (COTTON OR TEXAS) ROOT ROT IN ARIZONA. Ariz. Agr. Expt. Sta. Tech. Bul. 71, pp. [293]–410; illus.
(20) WALKER, J. C., and LARSON, R. H.
1935. CALCIUM CYANAMIDE IN RELATION TO CONTROL OF CLUBROOT OF CABBAGE. Jour. Agr. Res. 51: 183–189.
(21) ——— and MUSBACH, F. L.
1939. EFFECT OF MOISTURE, FERTILITY, AND FERTILIZER PLACEMENT ON ROOT ROT OF CANNING PEAS IN WISCONSIN. Jour. Agr. Res. 59: 579–590, illus.

EFFECT OF METAL CONTAINERS ON THE ANAEROBIC FERMENTATION OF CORNSTALK FLOUR BY THERMOPHILES[1]

By ROBERT P. STRAKA, *associate bacteriologist*, and G. H. NELSON, *assistant chemical engineer, Agricultural Byproducts Laboratory, Ames, Iowa* [2]

INTRODUCTION

The study reported in this paper was undertaken to ascertain the effect of metal containers on the continuous thermophilic anaerobic fermentation of cornstalk flour by a methane-producing seed. It was necessary to obtain this information before large metal tanks were built for the continuous anaerobic fermentation of fibrous farm wastes.

Much has been written on the oligodynamic action of heavy metals on micro-organisms, but the literature will not be reviewed here since Buchanan and Fulmer have given an excellent review in their book (*3*).[3]

MATERIALS EMPLOYED

Cornstalk flour was used as the test material because the authors had used it in previous experiments (*5*, *6*, *7*), and were familiar with the manner in which it is digested. It was prepared by wet-grinding the stalks in a rod mill and drying. Most of this flour passed a 200-mesh sieve.

The thermophilic methane-producing seed was originally developed from sewage sludge, as described in a previous publication (*5*). Before it was used, it was passed through a screen with circular openings 1 mm. in diameter, and all solids remaining on the screen were discarded. A preliminary analysis of the seed was made, and sufficient urea was added to bring the ammonia content of the mixture to from 500 to 800 p. p. m.

The fermentation apparatus consisted of wide-mouth glass bottles and metal containers. The glass bottles, used for controls, were fitted with three-hole rubber stoppers (No. 14), with outlets for the removal of samples for pH determinations, for the addition of chemicals when necessary, and for a gas exit. Anaerobic conditions were maintained by means of a water seal. Details of the construction of the glass fermentation apparatus are given in a previous publication (*5*). The gas was collected and measured in separate glass bottles. The 9-liter metal containers were made of copper, galvanized iron, black sheet iron, or stainless steel (18–8).[4] They were cylindrical with tapering tops, and the openings held the same size rubber stoppers as were used with the glass bottles. No solder was exposed on the inside of the containers. All details of the procedure were carried out the same in the metal as in the glass containers.

[1] Received for publication January 24, 1941.

[2] Operated by the Bureau of Agricultural Chemistry and Engineering in cooperation with the Iowa State College of Agriculture.

[3] Italic numbers in parentheses refer to Literature cited, p. 31.

[4] The stainless steel used was No. 24 gage with a No. 4 polish on one side. It had a Rockwell hardness of 82 to 87 on the B scale. The chemical analysis (percentage) was as follows: Chromium, 17.5 to 19; nickel, 8.0 to 9.0; carbon, 0.08 (approximate); manganese, 0.30 to 0.60; sulfur, 0.03 (maximum); and phosphorus, 0.03 (maximum).

Previous work in this laboratory (*5*) had shown that approximately half as much time was required to produce a unit quantity of gas from cornstalk flour at 50° to 55° C. as was required at 28° to 30°; therefore it was decided to employ the higher temperature in this experiment.

Five consecutive fermentations were run in all the containers except the copper, in which only four were run. In each run the following containers were used: Glass (seed only), glass (seed+cornstalk flour), galvanized iron, copper, black sheet iron, and stainless steel (18–8). The fermentations in the glass containers were controls. A detailed description of the preparation of the mixtures and the analyses for run 1 are given below. With the exception noted, the treatment of the mixtures was the same in all five runs.

PROCEDURE

Run 1.—With the exception of the seed control in the glass container, to which no cornstalk flour was added, all the fermentation mixtures were the same. They were prepared by placing 3 liters of seed, 1 liter of tap water, 65 gm. of cornstalk flour, and 2.5 gm. of urea in the container. The rubber stopper was tightly inserted, held securely with stovepipe wire, and sealed with shellac and sealing wax. The gas evolution tube was connected to a water seal, and the mixture was incubated at 50° to 55° C. for 22 days. The pH was checked frequently, and when necessary was adjusted to insure the optimum reaction (pH 7.0 to 8.0). A daily record was made of the gas produced. The volume of gas was measured as previously described (*5*), and the gas was analyzed for carbon dioxide, hydrogen, and methane by means of a modified Orsat apparatus (*4*).

At the termination of the 22-day fermentation period, all the containers were removed from the incubator, and the volume of the residue in each was measured. After thorough mixing, 1-liter aliquots were removed for analysis, and the remainder was returned to the container to serve as seed for the next run. A small part of one aliquot was used for a "wet" analysis; the remainder was evaporated to dryness on a steam hot plate, ground to pass a 30-mesh screen, and analyzed.

The following determinations were made on the wet samples: (1) Total solids; (2) ash-free solids (loss on ignition); (3) ash; (4) specific gravity; (5) ammonia nitrogen; (6) Kjeldahl nitrogen; and (7) metals (iron, copper, and zinc) according to the methods of the American Public Health Association and the American Water Works Association (*1*).

The following determinations were made on the dried residues: (1) Total solids; (2) ash-free solids (loss on ignition at about 600° C. for 1 hour) by the American Public Health Association and American Water Works Association method (*1*); (3) pentosans by the Association of Official Agricultural Chemists' method (*2*); (4) cellulose by the Norman and Jenkins' method (*8*) (with corrections made for ash and pentosans); (5) lignin by the Norman and Jenkins' method (*9*, *10*), substituting, however, 2-percent acid hydrolysis for 2 hours for the 5-percent hydrolysis normally used. (Duplicate lignin samples were run; one was ashed and the other was analyzed for Kjeldahl nitrogen by the A. P. H. A. and A. W. W. A. method (*1*).) A correction for ash was made, but no correction for nitrogen was applied, inasmuch as

Norman and Jenkins (*10*) have shown that the usual protein factor is not reliable); (6) Kjeldahl nitrogen by the A. P. H. A. and A. W. W. A. method (*1*); and (7) iron, copper, and zinc as follows: Iron by the thiocyanate method; copper by the sodium diethyldithiocarbamate method; and zinc by the ferrocyanide method as outlined by the American Public Health Association and American Water Works Association (*1*).

Run 2.—To the residue in each of the containers from run 1 were added 65 gm. of cornstalk flour, 1 liter of tap water, and ½ gm. of urea. No cornstalk flour was added to the seed control. The mixtures were incubated for 23 days. The rest of the procedure was the same as that outlined for run 1.

Run 3.—To each of the residues from run 2 were added the same quantities of cornstalk flour, water, and urea as were added in run 2. No cornstalk flour was added to the seed control. The mixtures were incubated for 25 days. At the end of the run, only a small sample (100 cc.) was removed for a "wet" analysis.

Run 4.—To each of the residues from run 3 were added 65 gm. of cornstalk flour and 1 gm. of urea. No water was added. No cornstalk flour was added to the seed control. The mixtures were incubated for 30 days. At the end of the run only a small sample was removed for a "wet" analysis.

Run 5.—To each of the residues from run 4 were added 65 gm. of cornstalk flour and 2 gm. of urea. No water was added. No cornstalk flour was added to the seed control. The mixtures were incubated for 60 days. At the end of the run a complete analysis was made (as in runs 1 and 2). The complete analysis of run 5 really was an analysis of the combined runs 3, 4, and 5.

RESULTS

As very little gas was produced in the seed control and the range of pH was optimal (pH 7.0 to 8.0), this part of the experiment will not be discussed further. In compiling the figures for the fermentations carried out in the other containers, a correction was made for the seed control.

For convenience the results are presented by "runs."

RUN 1

The volumes of gas produced are shown graphically in figure 1. The percentage decomposition of the constituents of the cornstalk flour, the composition of the gas, the pH range of the mixtures, and other pertinent data are presented in table 1. Table 6 shows the quantities of the metals in the mixtures at the end of the run.

In order to judge better the relative effects of the various metal containers on the fermentation, it was decided to rate the containers numerically on the basis of (1) the quantity of gas produced; (2) the methane content of the gas and the percentage decomposition of the (3) ash-free solids, (4) pentosans, (5) cellulose (corrected for ash and pentosans), and (6) lignin of the cornstalk flour. The following numerical values were assigned: 5 for maximum activity, 4 for next high, 3 for the third, 2 for the fourth, and 1 for the minimum activity. In case any two or more containers were rated approximately the same, each was given an equal share of the combined points for the two or more places in question. The maximum score obtainable would be 30.

TABLE 1.—*Decomposition of constituents in cornstalk flour fermented anaerobically for 22 days at 50° to 55° C. and gas produced, run 1*

Item	In original material			After fermenting for 22 days in—				
	Seed	Cornstalk flour	Total	Glass container	Galvanized iron container	Copper container	Sheet-iron container	18-8 stainless steel container
Constituents:								
Total solids ..... grams	63.25	62.97	[1] 128.72	87.65	94.53	123.32	89.01	85.70
Ash-free solids ..... do	41.36	60.74	[1] 104.60	62.22	67.81	95.03	61.68	58.90
Pentosans ..... do	1.54	20.58	22.12	5.41	6.50	17.08	5.63	4.64
Crude cellulose (ash-free) ..... do	3.30	29.69	32.99	9.76	10.50	23.42	8.63	7.01
Cellulose (corrected [2]) ..... do	2.63	17.61	20.24	7.48	7.89	18.75	6.68	5.40
Lignin ..... do	21.81	10.25	32.06	27.93	29.66	31.74	27.48	28.52
N^2 in lignin ..... percent	2.00	0.66	.....	1.95	1.98	1.73	2.04	1.83
Decomposition of constituents in cornstalk flour:								
Total solids ..... percent	.....	.....	.....	65.35	54.30	8.58	63.15	68.45
Ash-free solids ..... do	.....	.....	.....	69.75	60.90	15.75	70.55	75.20
Pentosans ..... do	.....	.....	.....	81.20	75.90	24.52	80.20	85.00
Crude cellulose (ash-free) ..... do	.....	.....	.....	78.20	75.80	32.22	81.80	87.50
Cellulose (corrected [2]) ..... do	.....	.....	.....	72.50	70.10	8.46	76.97	84.25
Lignin ..... do	.....	.....	.....	40.30	23.45	3.12	43.70	34.55
Gas produced under 30 inches of Hg at 60° F.:								
Total cc	.....	.....	.....	22,835	21,475	3,795	20,605	23,135
Per gram of cornstalk flour ..... do	.....	.....	.....	363	341	60	327	367
Per gram of ash-free solids in cornstalk flour cc	.....	.....	.....	376	354	63	339	381
Per gram loss of ash-free solids cc	.....	.....	.....	539	584	397	480	506
Average composition of gas:								
CO_2 ..... percent	.....	.....	.....	36.1	28.5	10.1	25.4	33.8
H_2 ..... do	.....	.....	.....	2.1	2.0	2.2	4.3	3.3
CH_4 ..... do	.....	.....	.....	45.4	43.3	0	49.6	45.7
pH range	.....	.....	.....	7.5–7.9	7.5–7.8	7.5–8.0	7.5–7.8	7.4–7.8

[1] Includes 2.5 gm. of urea.
[2] Corrected for pentosans and ash.

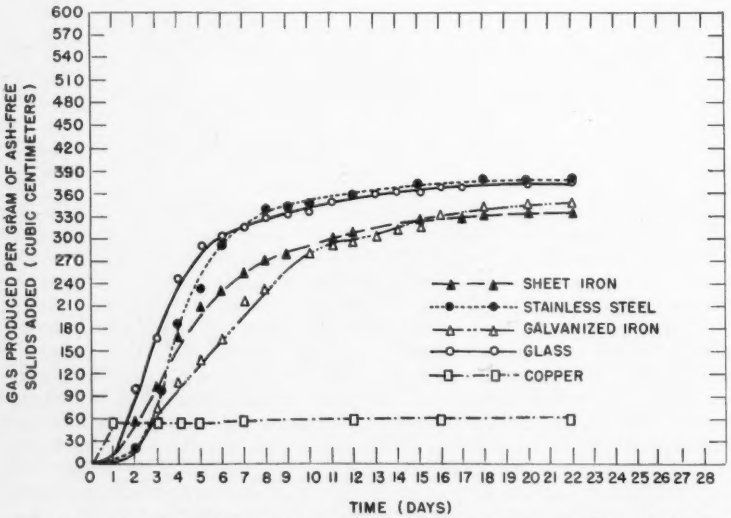


FIGURE 1.—Quantity of gas produced (cumulative) from a fermentation mixture of cornstalk flour and active methane-producing seed incubated at 50° to 55° C. in glass and various metal containers. Run 1.

The following example illustrates this method of evaluation: The volumes of gas produced (fig. 1) in the stainless-steel container and in the glass container were nearly the same but were greater than those produced in the other containers. Therefore, the numerical value given is $\frac{5+4}{2}$ or 4.5 points for each. Similarly, the methane values (table 1) for the stainless steel and for the glass containers were the same but were less than that for the sheet iron. The value $\frac{4+3}{2}$ or 3.5 is given for each. The ash-free solids losses (table 1) for the stainless steel, the glass, and the sheet-iron containers were fairly

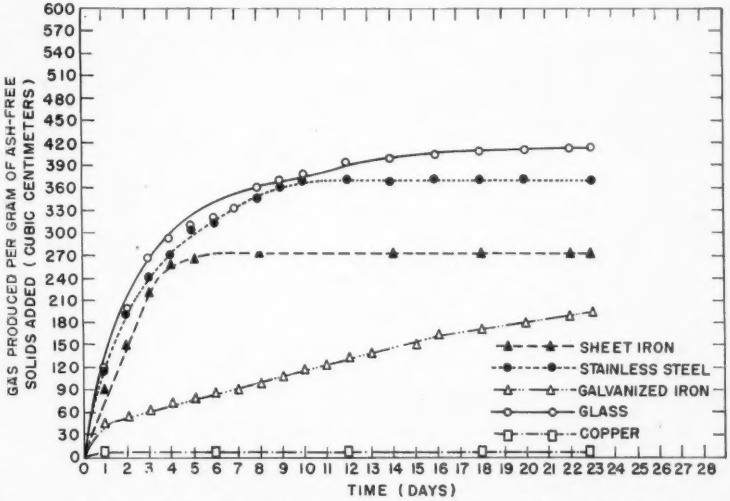


FIGURE 2.—Quantity of gas produced (cumulative) from a fermentation mixture of cornstalk flour and active methane-producing seed incubated at 50° to 55° C. in glass and various metal containers. Run 2.

close and were greater than those for the other containers. The value $\frac{5+4+3}{3}$ or 4 is given for each. The pentosan losses (table 1) for the stainless steel, the glass, and the sheet-iron containers were fairly close and were greater than those for the other containers. The value $\frac{5+4+3}{3}$ or 4 is given for each. The cellulose loss (table 1) was greatest for the stainless-steel container, and the value 5 is given. Lignin losses (table 1) were greater in the sheet-iron and the glass containers than in the stainless-steel container so the value 3 is given for the stainless steel. On this basis the containers were scored as follows: Stainless steel, 24; glass, 22.5; sheet iron, 21.5; galvanized iron, 16; and copper, 6.

RUN 2

The percentage decomposition of the constituents of the cornstalk flour, the composition of the gas, the pH range of the mixture, and other pertinent data are presented in table 2. The volumes of

the gas are shown graphically in figure 2. Table 6 gives the quantities of the metals in the mixtures at the end of the run.

The scoring, done on the same basis as in run 1, was as follows: Glass, 28; stainless steel, 21; sheet iron, 17.5; galvanized iron, 13.5; and copper, 10.

TABLE 2.—*Decomposition of constituents in cornstalk flour fermented anaerobically for 23 days at 50° to 55° C. and gas produced, run 2*

Item	At start in—					After fermenting for 23 days in—				
	Glass container	Galvanized-iron container	Copper container	Sheet-iron container	18-8 stainless-steel container	Glass container	Galvanized-iron container	Copper container	Sheet-iron container	18-8 stainless steel container
Constituents:										
Total solids.....grams..	[1] 128.34	[1] 133.95	[1] 159.39	[1] 129.50	[1] 127.76	87.13	106.53	142.97	93.03	92.10
Ash-free solids.....do....	[1] 107.29	[1] 111.80	[1] 132.35	[1] 107.00	[1] 105.42	62.12	80.20	111.24	64.89	66.50
Pentosans.....do....	24.58	25.43	33.37	24.76	24.06	6.54	12.24	26.74	7.27	7.06
Crude cellulose (ash-free) grams..	36.91	37.52	41.21	36.09	34.95	10.33	20.46	31.71	12.56	10.51
Cellulose (corrected [2]) do..	23.14	23.50	31.64	22.56	21.66	8.10	14.75	25.58	8.51	7.81
Lignin.....do..	30.92	32.38	34.00	30.63	31.65	26.49	29.13	28.58	28.02	28.65
N_2 in lignin...percent..						1.84	1.80	1.59	2.07	1.97
Decomposition of constituents in cornstalk flour:										
Total solids.....percent..						65.45	43.55	26.10	57.95	56.55
Ash-free solids.....do....						74.45	52.05	34.75	69.50	64.00
Pentosans.....do....						87.60	64.10	32.21	85.20	82.70
Crude cellulose (ash-free) percent..						89.45	57.45	31.92	75.80	82.45
Cellulose (corrected [2]) do..						85.45	49.60	34.40	79.80	78.55
Lignin.....do....						43.25	31.70	53.00	25.45	29.25
Gas produced under 30 inches of Hg at 60°F.:										
Total.....cc....						25,115	11,820	390	16,780	22,570
Per gram of cornstalk flour.....cc....						399	188	6.2	266	358
Per gram of ash-free solids in cornstalk flour.cc....						414	195	6.4	276	372
Per gram loss of ash-free solids.....cc....						556	374	18.5	399	580
Average composition of gas:										
CO_2.....percent..						39.1	24.1	2.0	29.9	33.2
H_2.....do....						1.9	1.5	2.9	1.8	2.2
CH_4.....do....						44.7	31.1	0	23.5	39.6
pH range.....						7.5–7.6	7.0–7.6	7.6–8.2	7.6–8.0	7.5–7.8

[1] From 65 gm. of cornstalk flour, 0.5 gm. of urea, and bacterial seed.
[2] Corrected for pentosans and ash.

RUNS 3, 4, AND 5

The volumes of gas are shown graphically in figures 3, 4, and 5. The percentage decomposition of the ash-free solids of the cornstalk flour, the pH range, the gas composition, and other pertinent data are recorded in table 3. Table 6 gives the quantities of the metals in the mixtures at the end of the runs. The scoring of the containers for these three runs was done on the basis of the quantity of gas produced, the methane content of the gas, and the percentage decomposition of the ash-free solids of the cornstalk flour. In run 3, with 15 as the highest score possible, the containers were rated as follows: Glass, 13; stainless steel, 12; sheet iron, 9; galvanized iron, 8; and copper, 3. In run 4, likewise with 15 as the highest score possible, the containers were rated as follows: Glass, 13; stainless steel, 12; sheet iron, 11; galvanized iron, 6; and copper, 3. In run 5, with 12 as the highest possible score, the containers were rated as follows: Stainless steel, 10.5; glass, 8.5; sheet iron, 8; and galvanized iron, 3.

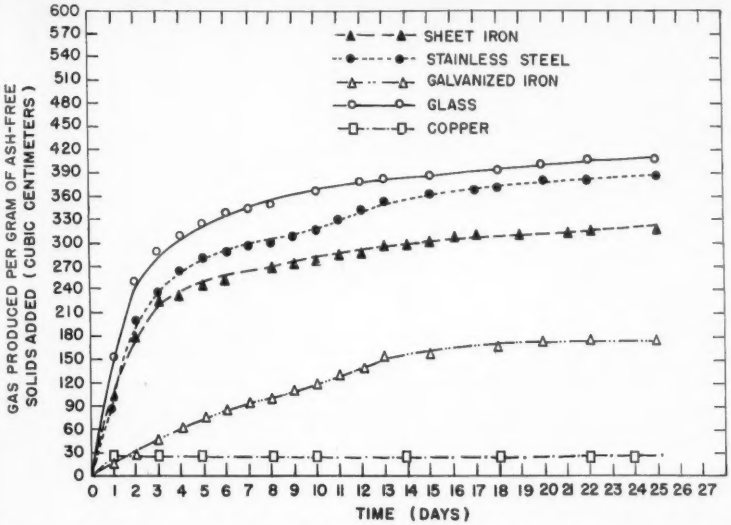


FIGURE 3.—Quantity of gas produced (cumulative) from a fermentation mixture of cornstalk flour and active methane-producing seed incubated at 50° to 55° C. in glass and various metal containers. Run 3.

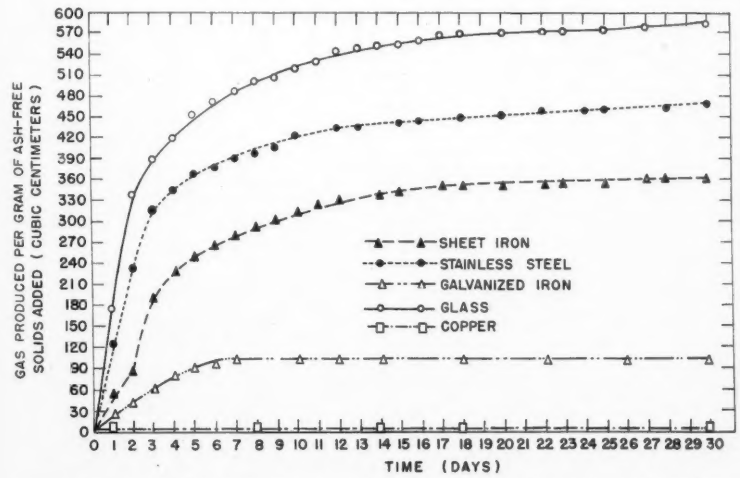


FIGURE 4.—Quantity of gas produced (cumulative) from a fermentation mixture of cornstalk flour and active methane-producing seed incubated at 50° to 55° C. in glass and various metal containers. Run 4.

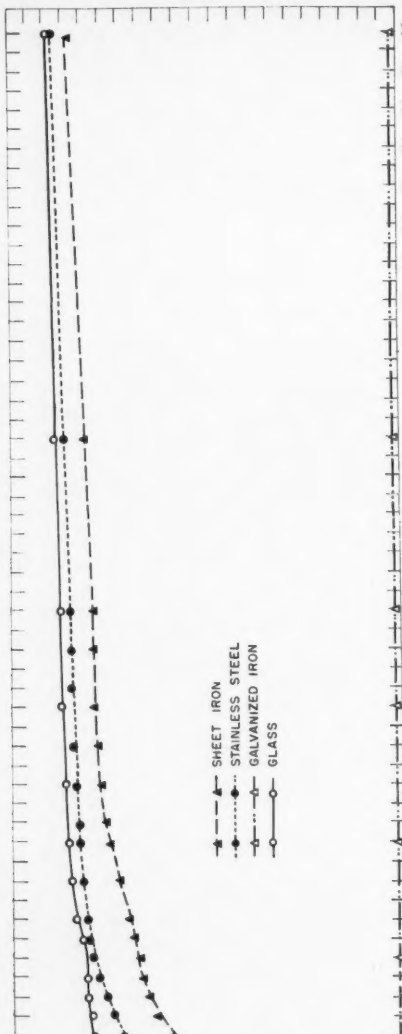


FIGURE 5.—Quantity of gas produced (cumulative) from a fermentation mixture of cornstalk flour and active methane-producing seed incubated at 50° to 55° C. in glass and various metal containers. Run 5.

TABLE 3.—*Decomposition of total and ash-free solids in cornstalk flour fermented anaerobically at 50° to 55° C. and gas produced, runs 3, 4, and 5 analyzed separately*

RUN 3 (FERMENTED FOR 25 DAYS)

Item	At start in—					After fermenting in—				
	Glass container	Galvanized-iron container	Copper container	Sheet-iron container	18-8 stainless-steel container	Glass container	Galvanized-iron container	Copper container	Sheet-iron container	18-8 stainless-steel container
Constituents:										
Total solids, grams	[1] 127.96	[1] 142.9	[1] 170.16	[1] 132.35	[1] 133.11	87.73	116.48	162.57	91.73	94.07
Ash-free solids, do	[1] 107.22	[1] 121.04	[1] 144.26	[1] 109.28	[1] 111.50	65.22	92.15	135.47	66.29	70.75
Decomposition of cornstalk flour:										
Total solids, percent						63.95	41.95	12.06	64.60	62.10
Ash-free solids, do						69.20	47.55	14.47	70.75	67.15
Gas produced under 30 inches of Hg at 60° F.:										
Total, cc						24,800	10,815	1,655	19,365	23,595
Per gram of cornstalk flour, cc						394	172	26	308	375
Per gram of ash-free solids in cornstalk flour, cc						408	178	27	319	389
Per gram loss of ash-free solids, cc						591	374	188	451	579
Average composition of gas:										
CO_2, percent						41.1	29.4	3.1	34.4	34.2
H_2, do						2.1	5.7	6.0	1.8	3.1
CH_4, do						50.6	51.1	3.8	44.5	52.6
pH range						7.4–7.8	7.1–7.6	7.4–7.6	7.2–8.0	7.3–7.8

RUN 4 (FERMENTED FOR 30 DAYS)

Item	At start in—					After fermenting in—				
	Glass container	Galvanized-iron container	Copper container	Sheet-iron container	18-8 stainless-steel container	Glass container	Galvanized-iron container	Copper container	Sheet-iron container	18-8 stainless-steel container
Constituents:										
Total solids, grams	[2] 148.27	[2] 176.01	[2] 220.39	[2] 152.08	[2] 154.48	98.79	159.18	216.75	103.26	107.72
Ash-free solids, do	[2] 124.40	[2] 150.37	[2] 194.42	[2] 125.41	[2] 129.82	74.74	131.89	185.62	75.27	82.33
Decomposition of cornstalk flour:										
Total solids, percent						78.60	26.70	5.79	77.55	74.35
Ash-free solids, do						81.81	30.40	14.48	82.45	78.20
Gas produced under 30 inches of Hg at 60° F.:										
Total, cc						35,415	6,295	230	21,935	28,260
Per gram of cornstalk flour, cc						562	100	3.7	348	449
Per gram of ash-free solids in cornstalk flour, cc						583	104	3.8	361	465
Per gram loss of ash-free solids, cc						713	341	26	438	595
Average composition of gas:										
CO_2, percent						39.8	16.9		24.4	31.0
H_2, do						0.5	0.9		1.2	1.6
CH_4, do						50.0	19.5		52.6	51.3
pH range						7.3–7.6	7.0–7.3	7.3–7.4	7.4	7.4

RUN 5 (FERMENTED FOR 60 DAYS)

Item	At start in—					After fermenting in—				
	Glass container	Galvanized-iron container	Copper container	Sheet-iron container	18-8 stainless-steel container	Glass container	Galvanized-iron container	Copper container	Sheet-iron container	18-8 stainless-steel container
Constituents:										
Total solids, grams	[3] 160.23	[3] 218.61		[3] 164.48	[3] 168.99	93.83	203.75		99.86	92.33
Ash-free solids, do	[3] 134.81	[3] 190.04		[3] 135.27	[3] 142.24	68.05	174.46		65.68	65.79
Decomposition of cornstalk flour:										
Total solids, percent						105.40	23.60		102.90	121.70
Ash-free solids, do						109.90	25.60		114.60	125.70
Gas produced under 30 inches of Hg at 60° F.:										
Total, cc						34,105	680		32,265	33,890
Per gram of cornstalk flour, cc						542	11		512	538
Per gram of ash-free solids in cornstalk flour, cc						562	11.2		531	558
Per gram loss of ash-free solids, cc						511	44		464	443
Average composition of gas:										
CO_2, percent						37.4			27.2	29.3
H_2, do						1.6			1.7	1.5
CH_4, do						52.7			56.0	55.2
pH range						7.4–7.6	7.0–7.1		7.2–8.0	7.4–7.6

[1] From 65 gm. of cornstalk flour, 0.5 gm. of urea, and bacterial seed.
[2] From 65 gm. of cornstalk flour, 1.0 of urea, and bacterial seed.
[3] From 65 gm. of cornstalk flour, 2.0 gm. of urea, and bacterial seed.

COMBINED RUNS NOS. 3, 4, AND 5

As previously mentioned, a complete analysis was made of the fermenting batches after runs 1 and 2, but not after runs 3 and 4. At the end of run 5 (after run 4 in the copper container), a complete analysis was again made. This constituted an analysis of the combined runs 3, 4, and 5. These data are presented in table 4.

TABLE 4.—*Decomposition of constituents in cornstalk flour fermented anaerobically at 50° to 55° C. and gas produced, runs 3, 4, and 5 combined* [1]

Item	At start in—					After fermenting for 121 days in—				
	Glass container [2]	Galvanized-iron container [2]	Copper container [2]	Sheet-iron container [2]	18–8 stainless-steel container [2]	Glass container	Galvanized-iron container	Copper container	Sheet-iron container	18–8 stainless-steel container
Constituents:										
Total solids.....grams	[3] 256.90	[3] 271.84	[4] 234.13	[3] 261.29	[3] 262.05	93.83	203.75	216.75	99.86	92.33
Ash-free solids.....do	[3] 231.70	[3] 245.52	[4] 206.00	[3] 233.76	[3] 235.98	68.05	174.46	185.62	65.68	65.79
Pentosans.....do	66.57	70.87	61.11	67.13	67.08	3.92	43.45	51.91	3.10	2.67
Crude cellulose (ash-free) grams	96.72	104.33	83.04	98.29	97.02	11.79	76.94	67.37	10.24	10.65
Cellulose (corrected [5]) grams	58.83	63.83	54.31	59.13	58.74	10.14	52.39	48.40	8.89	9.39
Lignin.....do	50.35	52.47	41.83	51.50	52.39	39.72	48.72	40.99	38.96	40.46
N_2 in lignin.....percent						2.48	2.90	2.16	2.86	2.61
Decomposition of constituents in cornstalk flour:										
Total solids.....percent						86.35	36.10	13.82	85.45	89.60
Ash-free solids.....do						89.75	39.01	16.78	92.25	94.25
Pentosans.....do						101.50	44.45	22.35	103.70	104.20
Crude cellulose (ash-free).....percent						95.20	30.75	26.40	98.80	96.95
Cellulose (corrected [5]) percent						92.20	21.65	16.78	95.00	93.45
Lignin.....do						34.55	14.50	4.05	40.80	38.75
Gas produced under 30 inches of Hg at 60° F.:										
Total.....cc						94,320	17,790	1,885	73,565	85,745
Per gram of cornstalk flour.....cc						499	94	15	389	454
Per gram of ash-free solids in cornstalk flour.cc						518	98	15.6	404	471
Per gram loss of ash-free solids.....cc						576	250	93	438	504

[1] Only 4 runs were made in the copper container.

[2] At the end of runs 3 and 4 a small sample (100 cc.) of the fermenting mixture was removed, and determinations were made only for total solids and ash-free solids. At the end of run 5 a liter sample (as in runs 1 and 2) was used for a complete analysis. Before beginning run 3, in addition to the cornstalk flour a liter of water was also added. This liter of water replaced the liter of fermenting mixture removed for a complete analysis. The addition of the water added a dilution factor which was taken into consideration when the figures in table 4 were computed. A complete analysis of the fermenting mixture in the copper container was made at the end of run 4.

[3] A total of 195 gm. of cornstalk flour and a total of 3.5 gm. of urea were added to the seed for runs 3, 4, and 5.

[4] A total of 130 gm. of cornstalk flour and a total of 1.5 gm. of urea were added to the seed for runs 3 and 4.

[5] Corrected for pentosans and ash.

The scoring of the containers was done on the same basis as in runs 1 and 2. With 30 as the highest possible score, the containers were rated as follows: Stainless steel, 24.5; glass, 24.0; sheet iron, 23.5; galvanized iron, 12.0; and copper, 6.0.

RATINGS BASED ON ALL RUNS

The sum of the scores for all runs, with 127 as the highest value possible, gives the following ratings: Glass, 109.0; stainless steel, 104.5; sheet iron, 90.5; galvanized iron, 58.5; and copper, 28.0. The scores are summarized in table 5.

TABLE 5.—*Summary of ratings of the various containers*

Run No.	Ratings for container made of—				
	Glass	Stainless steel	Sheet iron	Galvanized iron	Copper
1	22.5	24.0	21.5	16.0	6.0
2	28.0	21.0	17.5	13.5	10.0
3	13.0	12.0	9.0	8.0	3.0
4	13.0	12.0	11.0	6.0	3.0
5	8.5	10.5	8.0	3.0	.....
Combined 3, 4, and 5 [1]	24.0	24.5	23.5	12.0	[2] 6.0
Total	109.0	104.5	90.5	58.5	28.0

[1] Analysis of combined runs 3, 4, and 5 differs from that of the individual runs 3, 4, and 5 in that it includes the pentosans, cellulose, and lignin while the analyses of the individual runs do not.
[2] Does not include run 5.

QUANTITIES OF THE METALS IN THE FERMENTING MIXTURES AT THE END OF EACH RUN

The quantities of the metals present in the fermenting mixtures at the end of each run are shown in table 6.

TABLE 6.—*Quantities of metals in the fermenting solutions of cornstalk flour[1] in containers of different composition at the end of each run*

Type of container	Run No.	Quantity present at end of run			Type of container	Run No.	Quantity present at end of run		
		Iron	Copper	Zinc			Iron	Copper	Zinc
		P.p.m.	*P.p.m.*	*P.p.m.*			*P.p.m.*	*P.p.m.*	*P.p.m.*
Glass	1	314	.....	.....	Sheet iron	1	629	.....	.....
	2	440	.....	.....		2	1,915	.....	.....
	3	450	.....	.....		3	1,844	.....	.....
	4	459	.....	.....		4	1,825	.....	.....
	5	488	.....	.....		5	2,702	.....	.....
Galvanized iron	1	419	.....	100	Stainless steel	1	338	.....	.....
	2	509	.....	184		2	458	.....	.....
	3	818	.....	189		3	613	.....	.....
	4	773	.....	449		4	1,246	.....	.....
	5	974	.....	1,285		5	1,281	.....	.....
Copper	1	312	794	.....		At start	202	10	12.4
	2	421	894	.....		1	136	.....	.....
	3	421	996	.....	Seed control (glass)	2	78	.....	.....
	4	419	983	.....		3	71	.....	.....
						4	63	.....	.....
						5	66	.....	.....

[1] The cornstalk flour contained (on dry basis) per gram: 4.293 mg. of Fe, 0.01395 mg. of Cu, and 0.50 mg. of Zn.

In the interpretation of the results the following facts should be kept in mind: (1) At the beginning of runs 1, 2, and 3, a liter of water was added, thus causing a dilution factor; (2) a gram (dry basis) of the cornstalk flour contained 4.293 mg. of iron, 0.01395 mg. of copper, and 0.50 mg. of zinc (it is believed that a large part of this came from the equipment used for preparing the cornstalk flour); and (3) the seed contained some iron, copper, and zinc.

In general, there was an accumulation of iron in the fermenting masses of all containers with each succeeding run, the sheet-iron container showing the greatest increase and the copper and glass containers showing the least. At the end of run 5 (run 4 for the copper container), the quantity of iron in mixtures from the various contain-

ers was as follows: Sheet iron, 2,702 p. p. m.; stainless steel, 1,281 p. p. m.; galvanized iron, 974 p. p. m.; glass, 488 p. p. m.; and copper, 419 p. p. m. The seed control showed a decrease in iron from 202 p. p. m. at the beginning to 66 p. p. m. at the end of run 5.

Zinc determinations were made on the mixtures in the galvanized iron container at the end of each run. At the end of run 1, the zinc content was 100 p. p. m., and it gradually increased during each succeeding run to 1,285 p. p. m. at the end of run 5.

Copper determinations were made on the mixtures in the copper container at the end of each of the four runs. At the end of run 1 the copper content was 794 p. p. m., and it gradually increased to 983 p. p. m. at the end of run 4.

DISCUSSION

It would be possible to discuss the results from many angles, such as gas production, methane content, decomposition of the various constituents, and metallic content. The authors believe, however, that such an extensive discussion might confuse rather than help the reader. The primary object of the experiment was to ascertain the effect of metal containers on the continuous thermophilic anaerobic fermentation of fibrous farm wastes with repeated use of the same bacterial seed. It is believed that the criteria, including the method of rating, for judging the best type of metal container were fairly inclusive and reasonable.

The fermentations in the stainless-steel container compared favorably with those in the glass control. The quantities of gas produced in these two containers were fairly close throughout the five runs. The break-down of the various constituents, although it varied more widely than did the gas produced, was approximately of the same magnitude for the two containers. The cellulose and the pentosans were broken down the most readily; the lignin was the most resistant.

The results obtained with the sheet-iron container were rather erratic. Less gas was generally produced from the sheet-iron than from the glass container throughout the five runs. However, the relative quantities of gas produced from the sheet-iron container varied considerably from run to run, the smallest quantity being produced in run 2 and the largest in run 5. It is also interesting to note that, while less gas was produced in the sheet-iron container than in the glass, the breakdown of the various constituents was approximately of the same magnitude for both containers. Cellulose and pentosans were broken down the most readily, and lignin was the most resistant of the plant constituents.

The results obtained with the galvanized-iron and with the copper containers were rather unfavorable. Practically no gas was produced from the copper container throughout the four runs. Decreasing quantities of gas were obtained from the galvanized-iron container, and run 5 produced practically no gas. Greater quantities of the various constituents were broken down than could be accounted for by the gas produced throughout the runs in both the copper and the galvanized-iron containers.

SUMMARY

The effect of metal containers, as compared with glass, on the continuous thermophilic anaerobic fermentation of cornstalk flour by repeated use of a methane-producing seed was studied.

The stainless-steel (18–8) container was approximately as satisfactory as the glass container.

The results with the sheet-iron container were erratic and not so satisfactory as those obtained with the stainless steel.

The galvanized-iron and the copper containers were unsatisfactory.

LITERATURE CITED

(1) American Public Health Association and American Water Works Association.
1936. standard methods for the examination of water and sewage. Ed. 8, 309 pp., illus. New York.

(2) Association of Official Agricultural Chemists.
1935. official and tentative methods of analysis . . . compiled by the committee on editing methods of analysis. Ed. 4 710 pp., illus. Washington, D. C.

(3) Buchanan, R. E., and Fulmer, Ellis I.
1930. physiology and biochemistry of bacteria. V. 2, 709 pp. Baltimore, Md.

(4) Matuszak, Maryan P.
1934. unitized gas-analysis apparatus. Indus. and Engin. Chem., Analyt. Ed., 6: 72–78, illus.

(5) Nelson, G. H., Straka, Robert P., and Levine, Max.
1939. effect of temperature of digestion, chemical composition, and size of particles on production of fuel gas from farm wastes. Jour. Agr. Res. 58: 273–287, illus.

(6) ——— Straka, Robert P., and Levine, Max.
1939. decomposition and gas production of cornstalks under anaerobic conditions at 28° to 30° c. Iowa State Col. Jour. Sci. 13: 161–180, illus.

(7) ——— Straka, R. P., and Levine, Max
1940. anaerobic decomposition and gasification of cornstalks by thermophiles. Iowa State Col. Jour. Sci. 14: 233–251, illus.

(8) Norman, Arthur Geoffrey, and Jenkins, Samuel Harry.
1933. a new method for the determination of cellulose, based upon observations on the removal of lignin and other encrusting materials. Biochem. Jour. 27: [818]–831.

(9) ——— and Jenkins, Samuel Harry.
1934. the determination of lignin. I. errors introduced by the presence of certain carbohydrates. Biochem. Jour. 28: [2147]–2159.

(10) ——— and Jenkins, Samuel Harry.
1934. the determination of lignin. II. errors introduced by the presence of proteins. Biochem. Jour. 28: [2160]–2168.

INTERGENERIC HYBRIDIZATION OF CEREALS AND OTHER GRASSES[1]

By D. C. SMITH

Formerly *associate geneticist, Division of Forage Crops and Diseases, Bureau of Plant Industry, United States Department of Agriculture*

INTRODUCTION

Recent investigations with cereal and other grass species have indicated the possibility of obtaining new plant types by hybridization. Such crosses should be useful in eliminating certain undesirable characteristics possessed by many grasses otherwise very satisfactory agronomically. Improved yields of forage, either as pasture or hay, earliness, winter hardiness, ability to withstand drought, and higher grain yields have been claimed for certain segregates of wide crosses. Similarly, resistance of segregates to leaf and stem rusts, smuts, and mildew has been obtained. Susceptibility to shattering and lodging has been reduced.

Transfer of the generally larger size of the caryopsis in cereals to forage species would be of great practical value in reducing hazards and difficulty incident to seeding, such as accompany the culture of many common grasses. This is particularly true in wheat regions, where farmers unaccustomed to the necessity for unusual care in sowing light and small-seeded species often fail to obtain stands. Reduction or elimination of awn development would also facilitate seeding operations. Larger seeds could be much more easily threshed and processed. Increased grain yield and elimination of harsh characters such as awns might serve to increase feeding value and palatability.

Disadvantages of intergeneric hybrid progenies might be encountered in earlier generations at least. These would include comparative sterility, a probable lack of uniformity, perhaps susceptibility to shattering, and other weaknesses.

The forage improvement program has included extensive studies of exotic and endemic grass species and the application of basic breeding methods to these in an effort to obtain superior strains. Attention has been given to the possibilities of wide crosses as a means of supplementing the basic procedure. The investigations reported herein concern intergeneric hybridization of common wheat (*Triticum aestivum* L.), barley (*Hordeum vulgare* L.), and rye (*Secale cereale* L.) with other genera, principally in the tribe Hordeae, and of common oats (*Avena sativa* L. and *A. byzantina* C. Koch), chiefly with other members of the Aveneae. Descriptions of the hybrids are not given, since work with these is still in progress and only early generations have been grown.

REVIEW OF LITERATURE

WHEAT-GRASS CROSSES

The earliest and most extensive investigations on cereal-grass crosses have been reported by Russian workers. Much of the literature is not generally available. While some of the citations refer to

[1] Received for publication July 14, 1941. Cooperative investigations of the Division of Forage Crops and Diseases, Bureau of Plant Industry, and the Nursery Division, Soil Conservation Service, U. S. Department of Agriculture, and the Washington Agricultural Experiment Station.

Triticum durum Desf., these are considered to be pertinent to the present discussion. In some instances statements have been found to be quite general with insufficient detail as to specific parentage of hybrids.

The first *Triticum-Agropyron* hybrids known were made by Tzitzin in 1930, according to Armstrong (*4*).[2] Tzitzin (*30*) later reported, in 1933, that *Agropyron glaucum* Desf., *A. trichophorum* Link, *A. elongatum* (Host) Beauv., and *A. junceum* (L.) Beauv. would cross with wheat. This he again reported in 1940 (*35–a*). The F_1 hybrids of wheat and *A. elongatum* were characterized by a very vigorous root system and resistance to rust, smut, and mildew. They were also resistant to cold and drought. A large percentage of the second-generation plants were perennial. Some of these had luxuriant vegetative growth and were said to be promising as forage plants. In 1935 Tzitzin (*31*) confirmed the previous claims as to drought resistance and productivity of *Triticum-Agropyron* hybrids and indicated the yielding capacity of perennial forms to be increasing from year to year. F_2 plants varied greatly in fertility (*33*). In 1936 Tzitzin (*32*) stated that 60 to 70 percent of the F_4 generation were perennials and that constant forms having the wheat type of grain and the perennial habit had been isolated. Some of these were very early and many were more drought-resistant than wheat. In the same report Tzitzin announced the obtaining of hybrids between wheat and *Agropyron repens* (L.) Beauv. or a closely related form.

Vakar (*36*) attempted to cross *Triticum vulgare* (*T. aestivum*) and other wheat species with *Agropyron repens*, *A. tenerum* Vasey (*A. trachycaulum* (Link) Malte), *A. glaucum*, and *A. elongatum*. Hybrids were obtained only with *A. elongatum*, and the F_1 plants were completely sterile.

Veruschkine (*38*) stated that the cross *Triticum vulgare* × *Agropyron elongatum* was the most fertile of the wheat-*Agropyron* hybrids. Combinations were also reported to have been obtained between wheat and *A. elongatiforme* and *A. junceum*. Veruschkine and Shechurdine (*40*) reported that the hybrid seeds of *T. vulgare* × *A. elongatum* possessed apparently normal embryos and abortive endosperms, while seeds from *T. durum* × *A. elongatum* appeared to be normal.

Tzitzin (*34*) obtained perennial forms of the wheat type from *Triticum vulgare* var. *lutescens* × *Agropyron glaucum*. Armstrong (*4*) made crosses of two strains of *A. elongatum* and *A. glaucum* with three hexaploid and two tetraploid wheats. Attempts to combine *A. desertorum*, *A. dasystachyum* (Hook.) Scribn., *A. caninum* (L.) Beauv., *A. imbricatum* (M. B.) Roem. and Schult., *A. repens*, *A. cristatum* (L.) Gaertn. (commercial and Fairway strains), *A. obtusiusculum* Lange (*A. intermedium* (Host) Beauv.), and *A. richardsonii* Schrad. (*A. subsecundum* (Link) Hitchc.) with wheats were unsuccessful. *A. glaucum* combined more frequently with the tetraploids, while *A. elongatum* gave best results with hexaploid wheats.

Veruschkine and Shechurdine (*40*) crossed a pure line of *Triticum vulgare* var. *lutescens* with *Agropyron intermedium*. The latter species was found to cross readily with spring and winter wheats. Hybrids between *T. vulgare* and *A. intermedium* were sterile, while those having *T. durum* as the female parent were usually so. The seeds of the former cross possessed clearly developed embryos and usually aborted endosperms, while those of the latter appeared to be normal. Back-

[2] Italic numbers in parentheses refer to Literature Cited, p. 46.

crosses of *T. vulgare* × *A. intermedium* hybrids to wheat resulted in a low seed set. Raw (*27*) reported studies of hybrids between *T. vulgare* and *A. intermedium*.

Khižnjak (*13*) reported that *Triticum vulgare* × *Agropyron intermedium* hybrids formed unreduced egg cells previous to the production of sesquidiploids (derivatives from backcrossing) and triple hybrids. The same worker (*14*) referred to second and third generation hybrids of the cross *T. durum* × *A. intermedium*, though these were presumably the results of various backcrossing combinations. Some hybrids of 28-chromosome wheats with *A. intermedium* were self-fertile owing to the production of amphidiploid combinations. Khižnjak (*12, 13*) reported a triple hybrid from *T. durum* × *A. intermedium* pollinated by *T. vulgare*.

Vakar (*37*) summarized the results of cytological studies of F_1 to F_6 of *Triticum vulgare*×*Agropyron intermedium* (sterile)×*T. vulgare*, indicating that advanced generations had been obtained. Veruschkine (*39*) stated that some forms of *A. cristatum* cross with *T. timopheevizhuk*.

Longley and Sando (*19*) obtained crosses between Fulhio and Dawson × Kanred, varieties of *Triticum aestivum*, and *Secale montanum* Guss. In 1936 Deržavin (*7*) reported that two wheat varieties had been crossed with *Secale montanum*, and a fertile, perennial, hard wheat was obtained from the progeny. Guard (*9*) found that *T. aestivum* var. Chinese crossed readily with *S. montanum*.

Johnson (*10*) studied the compatibility of 27 forms of *Triticum* crossed with 18 species of *Agropyron*. The former included 17 varieties of *T. vulgare*, two wheat-rye hybrids, *T. durum*, *T. dicoccum* Schrank, two strains of *T. turgidum* L., *T. polonicum*, *T. persicum*, *T. timopheevi*, and *T. monococcum*. The *Agropyron* species used were *A. caninum*, *A. cristatum* (two strains), *A. dasystachyum*, *A desertorum*, *A. elongatum* (six strains), *A. glaucum*, *A. griffithsii*, *A. imbricatum*, *A. intermedium*, *A. junceum*, *A. obtusiusculum*, *A. pauciflorum*, *A. repens*, *A. richardsonii*, *A. sibiricum* (Willd.) Beauv., *A. smithii* Rydb., and *A. spicatum* (Pursh.) Scribn. and Smith. Johnson reported that *Agropyron elongatum* and *A. glaucum* were the only species crossing readily with *Triticum*, and these crossed only with *T. vulgare*, *T. durum*, and *T. dicoccum*. One plant of *T. persicum*×*A. junceum* was obtained. The seeds set ranged from 34 percent, in a cross between *A. glaucum* and one variety of *T. vulgare*, to 0, in several combinations of generally compatible crosses. The F_1 hybrids with *A. glaucum* were completely sterile, while a fair proportion were moderately fertile in the *A. elongatum* cross. Many germless seeds developed from certain combinations of parents. Germless seeds and stimulated ovaries were obtained from the use of *A. junceum*, *A. desertorum*, *A. imbricatum*, *A. sibiricum*, *A. intermedium*, and *A. dasystachyum*.

Crosses between spring and winter wheats and *Agropyron glaucum* and *A. trichophorum* were made by Nemlienko (*21*). More seeds set when these species were crossed with *Triticum durum* than with *T. vulgare*, and these were more viable in the first-named cross.

White (*42*) reported successful hybridization of nine varieties of *Triticum vulgare* with *Agropyron elongatum*. In crosses between the same wheat varieties and *A. glaucum*, seven set seed. Differences in seed setting were noted among the wheat parents, and hybrid seeds

from pollination by *A. glaucum* were only about one-third as numerous as when *A. elongatum* was used. Seeds were also better developed in the latter instance. Hybrids between *Triticum* species and *A. trichophorum* were reported.

In the United States, W. J. Sando, of the Division of Cereal Crops and Diseases, has worked extensively with wheat-*Agropyron elongatum* crosses, many hybrid combinations having been obtained.[3]

Michels [4] described a perennial hybrid between Mosida (*Triticum aestivum*) and *Elymus condensatus* Fresl., which was released in 1938 by the Idaho Agricultural Experiment Station. Poddubnaja-Arnoldi (*25*) obtained no normally developed seeds from crosses between *Triticum* and *Elymus* species, though in some instances embryos and endosperms began to be differentiated.

RYE-GRASS CROSSES

Crosses between *Secale cereale*×*S. montanum* were described by Kostoff (*15*) in 1931. The same hybrid was reported by Antonoff (*2*) and Deržavin (*7*) in 1936. Hybrids of *S. cereale* and other *Secale* species were discussed by Kostoff (*16*) in 1937. The F_1 of *S. cereale*× *S. montanum* was somewhat fertile, while F_1 plants of other combinations were highly fertile. Duka (*8*) in 1938 discussed work with Petkus rye crossed with *S. montanum* in 1930. Studies were made of F_2 to F_6 generations. In 1938 Rezničuk (*28*) reported making the same cross.

E. G. Schafer, of the Washington Agricultural Experiment Station, crossed *Secale cereale* with *S. montanum* in 1928. Several generations have been grown and selections have been made, primarily for grain but also for forage types. W. J. Sando has also made and studied this cross.[5]

Scattered reports of crosses of *Secale cereale* with other grass genera are available. Veruschkine and Shechurdine (*40*) obtained a hybrid seed set of 30.47 percent crossing *Secale cereale* × *Agropyron intermedium*. Crasniuk (*6*) in 1935 reported a hybrid between a fifth-generation inbred plant of a Russian rye, Eliseevskaja, and *Agropyron cristatum* (L.) P. B. var. *imbricatum* (M. B.) Moiss. This variety sometimes has been regarded as an independent species, *A. imbricatum*. One plant was obtained which proved to be a perennial. Pollen was almost completely sterile, and backcrosses were unsuccessful. Aldanov (*1*) and Veruschkine (*39*) likewise found that *S. cereale* and *A. cristatum* could be crossed successfully. In 1937 Ljubimova (*18*) reported the hybrid *S. cereale* × *A. glaucum* var. *genuinum*, which proved to be completely sterile. Tsitsin (*29*) also indicated that "rye-couch grass hybrids" had been obtained.

BARLEY AND OAT CROSSES WITH GRASSES

Very few workers have investigated hybridization of barley or oats with grasses. Malloch (*20*) pollinated *Hordeum vulgare pallidum* with *H. nodosum* L. but obtained no hybrid seeds. In crosses of *H. vulgare trifurcatum* × *H. "murianum"* (probably *H. murinum* L.), two viable seeds developed which resembled the maternal variety. After a retarded germination, seedlings died when 2 to 4 inches tall. Kuckuck (*17*) attempted to hybridize *H. sativum* and *H. bulbosum* L. With the

[3] Unpublished work.

[4] MICHELS, C. A. WHEAT-WILD RYE HYBRID GRASS. Idaho Agr. Expt. Sta. Leaflet 40, 4 pp. 1940. [Mimeographed.]

[5] Unpublished work.

latter variety as the female parent no seeds were obtained in 1,084 florets pollinated. In the reciprocal cross 4,578 florets of several varieties were pollinated. As a result 349 seeds were obtained, though these were only partially developed and usually were embryoless. One hybrid plant was obtained which upon examination proved to have 21 chromosomes in the somatic tissue. Rezničuk (*28*) attempted to cross *H. bulbosum* and *Elymus* species with cultivated barley but obtained no seeds. Quincke (*26*) has reported the crosses *H. jubatum* L. × *H. vulgare* and *H. nodosum* × *H. vulgare*.

No literature describing attempts to hybridize cultivated oats (*Avena sativa* or *A. byzantina*) with grasses of other genera has been seen by the author, though Johnson and McLennan (*11*) attempted unsuccessfully to cross the cultivated oat species with perennial types.

MATERIALS AND METHODS

The cereal parent was used as the female in all crossing combinations. Emasculation was accomplished by the use of forceps in the usual manner for small grains. Wheat spikes were reduced to 8 to 10 spikelets with 2 florets per spikelet. Barley heads were trimmed to 8 to 12 spikelets. Eight to 12 spikelets, each with 2 florets, were left on the rye rachis. Oat panicles were cut to 8 to 12 primary florets. Rye and barley florets were cut horizontally at about three-fifths of the length from the base to facilitate emasculation and pollination.

Inflorescences were covered with tissue-paper wrappings or parchment bags prior to pollination, which was accomplished 3 to 5 days after emasculation. In the case of rye it was done somewhat later. Pollinated heads were re-covered and left to mature.

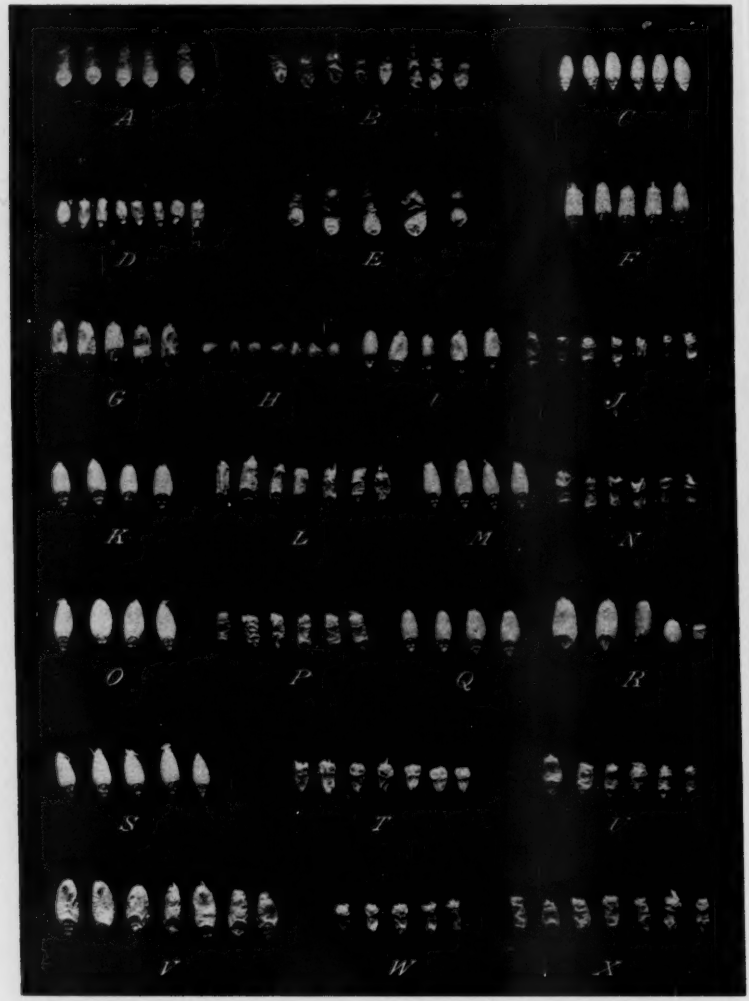
All of the seeds obtained in crossing operations were grown whether or not they appeared to be typical of the female parent. In some instances, particularly in rye, badly shrunken seeds produced normal plants.

In making hybrids, coincident production of receptive stigmas and fertile pollen is necessary. This was managed in the present studies by using several cereal varieties of each species, seeded at different dates. Subsequently the choice of a cereal parent was dependent only upon its being in the proper state of maturity when grass pollen was available.

Since the cereal varieties used in crossing are not indicated in the tabular summary, except for actual hybrids, they will be named to indicate the types utilized. Most of these bear identification numbers of the Division of Cereal Crops and Diseases of the Bureau of Plant Industry. The origin of others will be indicated briefly. All lots were previously grown at Pullman, Wash., by the Division of Cereal Crops and Diseases and the Washington Experiment Station.

Wheat varieties, in approximate order of frequency of use, were White Odessa-Hohenheimer, White Odessa-Heils Dickkopf, Ridit, Turkey-Florence, Regenerated Defiance, Federation, Marquis, Rex, Red Russian, Red May, Kinney, Redhart, and White Winter. White Odessa-Hohenheimer and White Odessa-Heils Dickkopf are strains derived by the Washington station from the corresponding crosses. Hybrid strains of the varieties Hohenheimer and Heils Dickkopf [6] are soft, white, late-winter wheats. Their extensive use was due primarily to their late maturity, coincident with flowering of grasses. Turkey-

[6] The varieties Hohenheimer and Heils Dickkopf were obtained by the Washington Experiment Station from Th. Roemer, of the University of Halle, Germany, in 1925.



Seeds of wheat varieties and of wheat varieties crossed with various grass species.

A, White Odessa-Heils Dickkopf. *B*, White Odessa-Heils Dickkopf × *Agropyron intermedium*. *C*, White Odessa-Heils Dickkopf. *D*, White Odessa-Heils Dickkopf × *Agropyron trichophorum*. *E*, White Odessa-Hohenheimer; result of pollination with grass pollen; not a hybrid. *F*, Kinney. *G*, Kinney × *Agropyron elongatum*. *H*, Unfertilized, pollinated White Winter wheat. *I*, Marquis. *J*, Marquis × *Agropyron repens*. *K*, White Winter. *L*, White Winter × *Agropyron intermedium*. *M*, White Odessa-Heils Dickkopf. *N*, White Odessa-Heils Dickkopf × *Agropyron intermedium*. *O*, White Odessa-Hohenheimer. *P*, White Odessa-Hohenheimer × *Agropyron intermedium*. *Q*, Regenerated Defiance. *R*, Regenerated Defiance × *Agropyron intermedium*. *S*, Red Russian. *T*, Red Russian × *Agropyron intermedium*. *U*, Marquis × *Agropyron trichophorum*. *V*, White Odessa-Hohenheimer × *Elymus condensatus*. *W*, White Odessa-Heils Dickkopf × *Agropyron sibiricum*. *X*, White Odessa-Heils Dickkopf × *Agropyron intermedium*.

Florence is a hard, white-grained, late-winter selection from the indicated cross.

Rye varieties included Rosen, Dakold, and a local spring strain known as Prolific. The varieties Beldi Giant, Winter Club, Orel, Tennessee Winter Smooth Awn, Tennessee Winter, Nobarb, and Eureka were used in barley-grass crosses. Markton, Gray Winter, and Red Rustproof were the oat varieties utilized in the few attempts made to obtain oat-grass crosses.

The grass strains used as male parents were pedigreed lots from native collections, foreign-plant introductions, and commercial sources. Chromosome numbers determined for these species by other workers are listed in table 1, as a descriptive feature of interest.[7] Since strains within species may vary widely in chromosome number, those given may be considered as suggestive only of the possible counts in the grass strains actually used, whose numbers have not been determined.

Investigations were begun in 1937 and continued, the present summary including the seasons from 1937 to 1939.

TABLE 1.—*Grass species used in crosses with chromosome numbers as reported by other investigators*

Species	Chromosome number ($2n$)	Authority and date	Reference
Agropyron caninum (L.) Beauv	28	Peto, 1930 [1]	(*41*)
Agropyron ciliare (Trin.) Franch	28	Nakajima, 1936	(*41*)
Agropyron cristatum (L.) Gaertn	14	Peto, 1930 [1]	(*24*)
Do	28	do [1]	(*41*)
Do	42	Araratian, 1938	(*3*)
Agropyron dasystachyum (Hook.) Scribn	28	Peto, 1930	(*41*)
Agropyron elongatum (Host) Beauv	70	do [1]	(*41*)
Agropyron inerme (Scribn. and Smith) Rydb			
Agropyron intermedium (Host) Beauv	42	Peto, 1930 [1]	(*41*)
Agropyron pungens (Pers.) Roem. and Schult	42	do [1]	(*41*)
Agropyron repens (L.) Beauv	42	Stolze, 1925 [1]	(*41*)
Agropyron semicostatum (Steud.) Nees	28	Nielsen and Humphrey, 1937 [1]	(*23*)
Agropyron sibiricum (Willd.) Beauv	28	Avdulow, 1928	(*41*)
Agropyron smithii Rydb	56	Peto, 1930 [1]	(*41*)
Agropyron spicatum (Pursh) Scribn. and Smith	14	do [1]	(*41*)
Agropyron trachycaulum (Link) Malte	28	do [1]	(*41*)
Agropyron trichophorum (Link) Richt	42	Araratian, 1938	(*3*)
Arrhenatherum elatius (L.) Mert. and Koch	28	Aase and Powers, 1926 [1]	(*41*)
Bromus carinatus Hook. and Arn	56	Stählin, 1929	(*41*)
Bromus inermis Leyss	42	do	(*41*)
Do	56	Avdulow, 1928	(*41*)
Do	56–70	Nielsen, 1939	(*22*)
Dactylis glomerata L	28	Davies, 1927 [1]	(*41*)
Elymus ambiguus Vasey and Scribn. (var. *strigosus*	28	Nielsen and Humphrey, 1937	(*23*)
Elymus angustus Trin			
Elymus arenarius L			
Elymus canadensis L	28	Avdulow, 1928 [1]	(*41*)
Do	42	Nielsen and Humphrey, 1937	(*23*)
Elymus condensatus Presl	28	do	(*23*)
Elymus giganteus Vahl	28	Avdulow, 1931	(*41*)
Elymus glaucus Buckl			
Elymus junceus Fisch			
Elymus triticoides Buckl			
Festuca elatior L	14	Stolze, 1925	(*5*)
Festuca idahoensis Elmer			
Hordeum brevisubulatum (Trin.) Link			
Hordeum bulbosum L	28	Stählin, 1929 [1]	(*41*)
Hordeum jubatum L	14	Tanji, 1925	(*41*)
Do	28	Aase and Powers, 1926 [1]	(*41*)
Hordeum nodosum L	14	Tanji, 1925	(*41*)
Do	42	Griffee, 1927	(*41*)
Koeleria cristata (L.) Pers	70	Avdulow, 1931	(*41*)
Lolium perenne L	14	Evans, 1926 [1]	(*41*)
Secale montanum Guss	14	Stolze, 1925 [1]	(*5*)

[1] Substantiated by other investigators.

[7] Since this manuscript was prepared, Stebbins and Love (*29*) have reported $2n$ numbers to be *Elymus glaucus* 28, *E. triticoides* 42, and *Festuca idahoensis* 28. Counts for several other species listed in table 1 were verified. The $2n$ number for *Koeleria cristata* was found to be 28.

RESULTS

In the discussion of results a description of any hybrids obtained will be omitted, since work with these is being continued. It may be stated, however, that F_1 plants have proved to be completely sterile in all crosses studied except that of *Secale cereale* × *S. montanum*.

WHEAT-GRASS CROSSES

Data summarizing the results with hybrids having wheat as the female parent are listed in table 2. Columns 3 and 4 indicate the number of different wheat varieties and grass strains used with each grass species. The numbers of seeds indicate only those seeds that appeared to be hybrid or later produced hybrid plants. Occasional selfed seeds were found, but these were eliminated from the summary. Emasculated unpollinated checks set less than 0.5 percent of seeds. It appeared that during the time the covering was removed for pollination stray pollen became effective.

Many seeds, possibly of hybrid origin, failed to germinate. Others, undoubtedly hybrid, also failed to grow. In plate 1, *E*, *V*, and *W*, deformed seeds that failed to grow or were not hybrid are shown. Some seeds appeared to be without embryos, e. g., the two on the right in plate 1, *E*. Normal grains of the same variety are shown in plate 1, *A*, and pollinated, unfertilized ovaries may be seen in plate 1, *H*.

TABLE 2.—*Summary of results of artificial pollination in attempted hybridization of common wheat (Triticum aestivum) with other grass species*

Grass species	Period of study	Wheat varieties	Grass strains	Total florets	Hybrid seeds	Hybrid plants
	Years	*Number*	*Number*	*Number*	*Number*	*Number*
Agropyron caninum	1	2	2	534	0	0
Agropyron ciliare	1	2	1	81	0	0
Agropyron cristatum	3	4	4	882	1	1
Agropyron dasystachyum	3	3	1	80	3	0
Agropyron elongatum	1	2	1	332	17	11
Agropyron inerme	3	4	2	701	0	0
Agropyron intermedium	1	5	2	2,408	363	20
Agropyron pungens	1	2	1	426	0	0
Agropyron repens	2	1	3	720	0	0
Agropyron semicostatum	1	1	1	140	0	0
Agropyron sibiricum	1	5	1	578	0	0
Agropyron smithii	2	3	1	356	0	0
Agropyron spicatum	1	3	1	716	0	0
Agropyron trachycaulum	2	4	2	710	0	0
Agropyron trichophorum	3	7	1	1,128	76	16
Elymus angustus	1	1	1	416	0	0
Elymus canadensis	2	2	4	702	0	0
Elymus condensatus	3	5	4	1,619	0	0
Elymus giganteus	1	2	2	120	0	0
Elymus glaucus	1	2	2	134	0	0
Elymus junceus	1	1	1	18	0	0
Elymus triticoides	2	2	1	785	0	0
Festuca elatior	2	4	2	452	0	0
Festucaidahoensis	1	1	1	74	0	0
Lolium perenne	1	1	1	40	0	0
Secale montanum	2	2	1	610	0	0

One dwarfed seedling was derived from a cross of Rex × *Agropyron cristatum*. This plant produced five leaves before premature death. Since numerous segregating progenies of crosses between *Triticum aestivum* and *A. elongatum* have been available through the work of W. J. Sando, attempts to obtain new local combinations of this cross have not been extensive. Eleven plants from 17 hybrid seeds resulted from pollination of 286 florets of Kinney × *Agropyron elongatum*.

The nature of seeds producing hybrid plants is shown in plate 1, *G*, and the female parent in plate 1, *F*.

During the period of investigation, combinations of one wheat variety with three strains of *Agropyron repens* have been attempted. The strains of *A. repens* were used only with White Odessa-Hohenheimer, and all failed to cross with this wheat.

Fourteen hybrid plants were obtained from the cross of White Odessa-Heils Dickkopf × *Agropyron intermedium*. Hybrid seeds are shown in plate 1, *B* and *X*. Similar hybrid seeds were produced by crossing White Odessa-Hohenheimer, Red Russian, White Winter, and White Odessa-Heils Dickkopf with a shorter, finer form of *A. intermedium* resembling *A. repens*. Five seeds produced with Regenerated Defiance as a parent are shown in plate 1, *R*, plate 1, *Q*, being the normal seeds of Regenerated Defiance. The two small seeds at the right were peculiar and unlike each other or the female parent. Neither of these seeds produced hybrid plants. The three other seeds proved to be the result of selfing. Though 260 florets of Marquis were used no seeds were obtained. The percentages of shrunken seed set by the respective wheat varieties were White Odessa-Hohenheimer, 34; White Odessa-Heils Dickkopf, 18; Red Russian, 12; White Winter, 4; Marquis, 0; and Regenerated Defiance, 0. Plate 1, *A* to *T*, inclusive, illustrates the types of seeds obtained from the crosses, as compared with those of the female parents.

Six plants were obtained from wheat × *Agropyron intermedium* (short form) crosses, all coming from White Odessa-Hohenheimer used as the female parent. Incidentally this cross gave the highest percentage of seed set, as previously indicated. Seeds that produced plants were classified as either "badly shrunken" or "very badly shrunken."

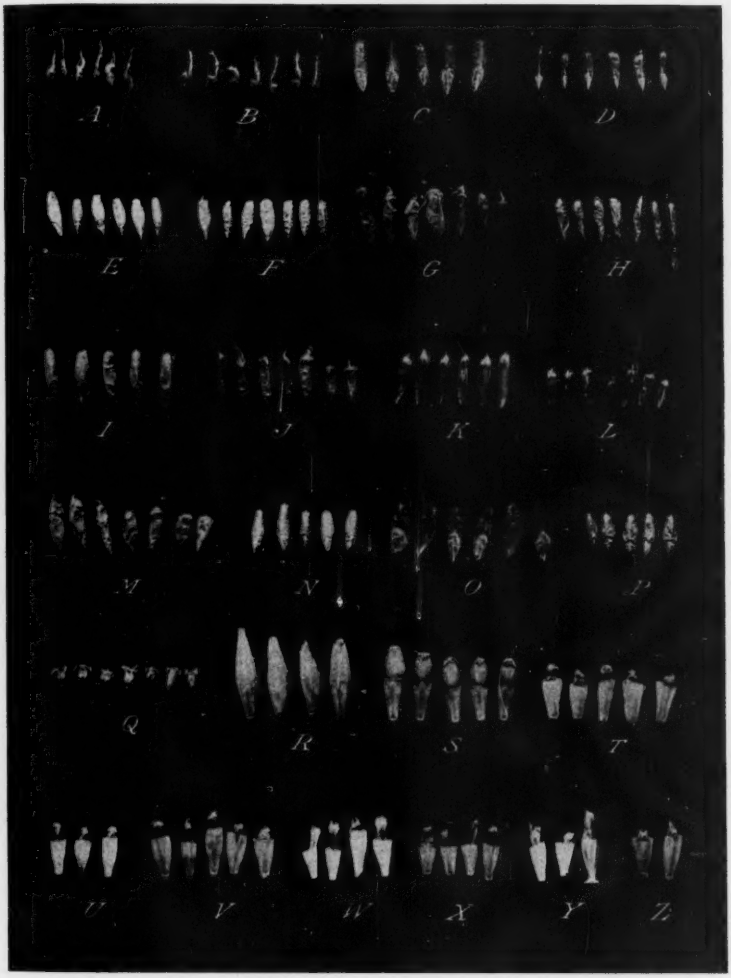
Red Russian, Ridit, Regenerated Defiance, Marquis, White Odessa-Heils Dickkopf, White Odessa-Hohenheimer, and Federation, the latter 2 in a very limited way, were used in crosses with *Agropyron trichophorum*. In 1,128 florets of 7 wheat varieties, 76 seeds were obtained; 16 of these produced plants. Seeds of this cross, with the female parents, are shown in figure 1, *C*, *D*, *I*, and *U*. White Odessa-Heils Dickkopf was the most successful parent, though use of Red Russian and Marquis also resulted in hybrid seeds. Regenerated Defiance and Ridit, though more extensively used than other varieties, did not function successfully as parents.

All of the seeds obtained in crossing operations were grown whether or not they appeared to be typical of the female parent. In some instances, particularly in rye, badly shrunken seeds produced normal plants.

No crosses other than those previously discussed and indicated in table 1 were obtained from pollinations of wheat varieties.

RYE-GRASS CROSSES

Summary data indicating results of attempted crosses with rye as the female parent are given in table 3. In all, 19 species of grass comprising 27 strains were used with Rosen, Dakold, and Prolific ryes. Many seeds were obtained, most of which proved to be normal rye when resulting plants were observed.



Seeds of cereal varieties and of cereal varieties crossed with various grass species.

A–P, Rye varieties and crosses: *A*, Rosen rye artificially pollinated, unfertilized; *B*, Prolific rye artificially pollinated, unfertilized; *C*, Prolific rye; *D*, Prolific rye × *Agropyron repens*; *E*, Dakold; *F*, Dakold × *Secale montanum*; *G*, Prolific rye × *Agropyron sibiricum*; *H*, Prolific Rye × *Secale montanum*; *I*, Prolific rye; *J*, Prolific rye × *Agropyron trichophorum*; *K*, *Secale montanum*; *L*, Prolific rye × *Secale montanum*; *M*, Rosen × *Agropyron trachycaulum*; *N*, Prolific rye; *O*, Prolific rye × *Elymus glaucus*; *P*, Prolific rye × *Elymus condensatus*. *Q–Z*, Barley varieties and crosses: *Q*, Beldi Giant barley, artificially pollinated, unfertilized; *R*, Beldi Giant barley; *S*, Beldi Giant barley, pollinated, fertilized; *T*, Beldi Giant barley × *Secale montanum*; *U*, Tennessee Winter × *Hordeum bulbosum*; *V*, Orel × *Hordeum bulbosum*; *W*, White Winter × *Agropyron trichophorum*; *X*, Nobarb × *Agropyron cristatum*; *Y*, White Winter × *Secale montanum*; *Z*, White Winter × *Hordeum nodosum*.

TABLE 3.—*Summary of results of artificial pollination in attempted hybridization of common rye (Secale cereale) with other grass species*

Grass species	Period of study	Rye varieties	Grass strains	Total florets	Hybrid seeds	Hybrid plants
	Years	*Number*	*Number*	*Number*	*Number*	*Number*
Agropyron cristatum	2	3	3	942	0	0
Agropyron dasystachyum	1	1	1	60	0	0
Agropyron pungens	1	1	1	24	0	0
Agropyron repens	1	1	1	220	43	16
Agropyron sibiricum	2	1	1	1,230	1	1
Agropyron smithii	2	2	1	486	0	0
Agropyron spicatum	2	3	1	1,486	0	0
Agropyron trachycaulum	1	2	2	444	0	0
Agropyron trichophorum	2	3	1	1,434	197	17
Bromus inermis	1	1	1	130	0	0
Elymus ambiguus	1	1	1	124	0	0
Elymus canadensis	2	1	4	1,050	0	0
Elymus condensatus	2	3	2	1,058	0	0
Elymus giganteus	1	1	1	552	0	0
Elymus glaucus	2	1	1	266	0	0
Elymus junceus	1	2	1	106	0	0
Elymus triticoides	1	1	1	602	0	0
Hordeum bulbosum	2	2	1	160	0	0
Secale montanum	1	1	1	664	207	175

Sixteen plants were obtained from 43 seeds of the cross Prolific rye × *Agropyron repens*. Hybrid seeds and those of the rye parent are shown in plate 2, *C* and *D*. Plate 2, *A* and *B*, may be used to compare seed development with ovaries of pollinated, unfertilized Rosen and Prolific ryes. While the hybrid seeds were badly shrunken, a good percentage produced plants. As indicated in table 3, attempts were not made to cross *A. repens* with Rosen and Dakold.

One plant was obtained from the cross Prolific rye × *Agropyron sibiricum*, 1,230 florets being manipulated. Though many seeds were produced, most of these were not crosses. The seed from which the plant was derived was badly shrunken, as shown in plate 2, *G*.

Pollination of 1,064 florets of Prolific rye with *Agropyron trichophorum* resulted in seeds producing 17 plants of hybrid nature. Though 197 hybrid seeds were listed as obtained (table 3), many of these were shrunken and yet were not hybrid. Actual germination of hybrid seeds is, therefore, problematical. Seeds of this cross are shown in plate 2, *J*, the Prolific rye parent being indicated in plate 2, *I*. Though 278 florets of Rosen and 92 of Dakold were pollinated with this species, no hybrid plants were obtained.

Crosses of *Secale cereale* × *S. montanum* were obtained with relative ease. The percentages of success in using Rosen and Dakold were similar, with Prolific rye somewhat lower but perhaps not significantly so. Hybrid seeds of this cross are shown in plate 2, *F*, *H*, and *L*, for Dakold and Prolific rye derivatives. Those from crosses with Rosen were similar in appearance. A very high percentage of the seeds of this cross were viable, and 175 hybrid plants were obtained. The common rye varieties were standard lots locally grown previous to use as parents. *S. montanum* was obtained from stray plants growing on the Washington Experiment Station farm. The nature of the seeds of this species is shown in plate 2, *K*.

Though other crosses of ryes with grasses were attempted, none was successful. Many seeds obtained from attempted control of pollination proved to be due to selfing or to accidental crossing when the spikes were uncovered for pollination. The extent of development of the endosperm in such caryopses is indicated in plate 2, *M*, *O*, and *P*.

Examination of pollen of the supposed F_1 plants was resorted to in some rye crosses to aid in determining whether plants resulting from crossing were actual hybrids. It was first supposed, however, that the rye parent plants would exhibit a fairly high, reasonably uniform percentage of fertile pollen. In 56 plants of Prolific rye from greenhouse-grown cultures, of which anthers were examined, none showed entirely normal pollen. In some anthers pollen had aborted completely. Many plants showed 20 to 50 percent of pollen aborted. Examination of the contents of 3 anthers from 1 floret and several florets per spike indicated wide differences, not to be considered as due to occasional aborted or partially aborted anthers in otherwise normal florets. Pollen examination as a preliminary method of determining hybrid origin was found not to be helpful in this material, since the plants examined were not hybrids, as was later proved.

BARLEY-GRASS CROSSES

Data concerning attempted hybridization of common barley with grasses are shown in table 4. No hybrid seeds sufficiently developed to produce plants were obtained. Development of the ovary was stimulated by application of pollen from certain species, principally *Hordeum bulbosum*. In plate 2, *Q*, are shown ovaries of Beldi Giant pollinated but unfertilized. Plate 2, *R* and *S*, shows normal Beldi Giant and kernels as developed after clipping of the lemma in emasculation. Plate 2, *T*, indicates the degree of development of the caryopsis following crossing with *Secale montanum*. Similar development is shown for crosses of Tennessee Winter×*Hordeum bulbosum* (pl. 2, *U*), and Orel×*Hordeum bulbosum* (pl. 2, *V*), though one seed of the latter is further developed and proved to be due to selfing. Plate 2, *W* to *Z*, indicates the development of the caryopsis when several other grass parents were utilized for pollen. Some of the better developed seeds grew, these proving to be selfs. It is evident in comparing plate 2, *T* to *Z*, with plate 2, *Q*, that partial development occurred in the former.

TABLE 4.—*Summary of results of artificial pollination in attempted hybridization of common barley (Hordeum vulgare) with other grass species*

Grass species	Period of study	Barley varieties	Grass strains	Total florets	Hybrid seeds	Hybrid plants
	Years	*Number*	*Number*	*Number*	*Number*	*Number*
Agropyron cristatum	3	3	4	379	0	0
Agropyron intermedium	1	1	1	143	0	0
Agropyron repens	1	3	1	127	0	0
Agropyron smithii	2	2	1	431	0	0
Agropyron trichophorum	1	1	1	67	0	0
Bromus carinatus	1	1	1	30	0	0
Bromus inermis	1	2	2	183	0	0
Elymus ambiguus	1	1	1	94	0	0
Elymus angustus	1	1	1	91	0	0
Elymus arenarius	1	1	1	57	0	0
Elymus canadensis	1	1	2	131	0	0
Elymus condensatus	2	2	3	185	0	0
Elymus giganteus	2	2	1	274	0	0
Elymus glaucus	2	2	2	167	0	0
Elymus junceus	1	1	1	101	0	0
Elymus triticoides	3	5	2	320	0	0
Festuca idahoensis	1	1	1	94	0	0
Hordeum brevisubulatum	1	2	1	289	0	0
Hordeum bulbosum	2	3	1	698	0	0
Hordeum jubatum	1	1	1	281	0	0
Hordeum nodosum	2	3	1	402	0	0
Secale montanum	2	2	1	813	0	0

OAT-GRASS CROSSES

Attempts to hybridize cultivated oats with grass species have been very limited except with *Arrhenatherum elatius*. The oat varieties used were Markton, Red Rustproof, and Winter Turf; all were used with *A. elatius*. A summary of the results is given in table 5. No seeds appearing to vary from the normal were observed, and no crosses were successful.

TABLE 5.—*Summary of results of artificial pollination in attempted hybridization of common oats (Avena sativa and A. byzantina) with other grass species*

Grass species	Period of study	Oat varieties	Grass strains	Total florets	Hybrid seeds	Hybrid plants
	Years	*Number*	*Number*	*Number*	*Number*	*Number*
Arrhenatherum elatius	2	3	1	411	0	0
Bromus inermis	1	1	1	43	0	0
Dactylis glomerata	1	1	1	96	0	0
Elymus canadensis	1	1	1	68	0	0
Koeleria cristata	1	1	1	28	0	0

DISCUSSION

Various workers, including Armstrong (*4*), Veruschkine and Shechurdine (*40*), and Kikot and Volkova, according to Tzitzin (*35*), have indicated that success in crossing depends primarily on the parental strains used. Armstrong found two *Agropyron elongatum* types to be quite different in compatibility with wheat. Kikot and Volkova stated further that individual plants within a strain might vary greatly in this respect. The present study has indicated substantiation of this for the female parents. Similar differences in relative compatibility of male parents might be expected to exist. Negative results from attempted crosses should be regarded, therefore, as applying only to the strains used, and not as establishing the complete incompatibility of the species.

In consideration of results of investigations, possible differences in botanical nomenclature should be recognized. While for certain species agreement in identity may be assumed, this would not apply in all instances. The relation of *Agropyron intermedium*, *A. obtusiusculum*, and *A. glaucum* may be synonymous. However, the *A. glaucum* strain used by Armstrong (*4*) and Johnson (*10*) is not thought to be closely similar to the *A. intermedium* used by the present writer. Specific distinction in such polymorphic forms as *A. repens* is also subject to much individual interpretation.

The results of crossing are in general agreement with those previously reported. All of the wheat-grass crosses obtained have been made by other workers. The possible compatibility of certain races of *Agropyron repens* with *Triticum aestivum* was indicated by Tzitzin (*32*), though again attempts to make this cross have been unsuccessful. In the present studies *T. aestivum* and *S. montanum* were not compatible. The influence of parental strains utilized suggests itself as a possible factor in the results obtained.

So far as the writer knows the crosses *Secale cereale* × *Agropyron repens*, *S. cereale* × *A. sibiricum*, and *S. cereale* × *A. trichophorum* have not been reported in earlier literature. The hybrid *S. cereale* × *A. intermedium* obtained by Veruschkine and Shechurdine might be very

similar to the last-named cross, however, since *A. trichophorum* is considered by these workers to be closely related to *A. intermedium*. The hybrid of Ljubimova (*18*) might be considered in this group. Tzitzin (*35–a*) also referred to "rye-couch grass hybrids." Intergrading biotypes between *A. intermedium* and *A. repens* may also exist. The fact that the three species previously mentioned have been reported to have the same chromosome number conforms with observed morphological similarities as a basis for their possibly close relationship.

While no grass species were crossed successfully with *Hordeum vulgare*, the partial development of the barley caryopsis following pollination with certain ones suggests that some incomplete compatibility may exist. This development was particularly noticeable in the results of pollinating *H. vulgare* × *H. bulbosum* but was also observed in other hybrid combinations. The recent results of Quincke (*26*) suggest the problem of the relative merits of reciprocal crosses in obtaining hybrids successfully.

SUMMARY

Hybridization of wheat (*Triticum aestivum* L.) was attempted with other grasses, including 15 species of *Agropyron*, 7 of *Elymus*, 2 of *Festuca*, *Lolium perenne*, and *Secale montanum* as pollen parents.

Certain hybrid plants were obtained only in the following crosses: *Triticum aestivum* × *Agropyron elongatum*; *T. aestivum* × *A. intermedium*; and *T. aestivum* × *A. trichophorum*.

Rye varieties (*Secale cereale* L.) were studied in combinations with nine species of *Agropyron*, seven of *Elymus*, and *Bromus inermis*, *Hordeum bulbosum*, and *Secale montanum*. The following crosses were successful: *Secale cereale* × *Agropyron repens*, *S. cereale* × *A. sibiricum*, *S. cereale* × *A. trichophorum*, and *S. cereale* × *S. montanum*.

Five *Agropyron*, two *Bromus*, nine *Elymus*, and four *Hordeum* species, and *Festuca idahoensis* and *Secale montanum* were used as male parents in crosses with *Hordeum vulgare* L. No hybrids were obtained.

Limited attempts to hybridize cultivated oats (*Avena sativa* L. and *A. byzantina* C. Koch) with other grasses, particularly *Arrhenatherum elatius*, were unsuccessful.

Hybrid plants studied have proved to be sterile in all crosses except those derived from *Secale cereale* × *S. montanum*.

All varieties used in wheat × grass and rye × grass crosses were not equally successful, results varying with the strains utilized as female parents. Inability to produce particular strain combinations between species should not be considered as proof of complete incompatibility of the species in intergeneric crosses.

LITERATURE CITED

(1) Aldanov, A. D.
1936. [25 years work of saratov central station of plant-breeding and genetics.] Sotsialist. Zern. Khoz. (Grain Prod. Jour.) No. 2, pp. [131]–142, illus. [In Russian. Summary in Plant Breeding Abs. 7 (85): 21. 1936.]

(2) Antonoff, Slaw.
1936. beitrag zum zytogenetischen studium der artbastarde triticum turgidum × triticum durum, triticum durum × triticum monococcum und secale cereale × secale montanum. Züchter 8: 240–243 illus.

(3) ARARATIAN, A. G.
1938. [THE CHROMOSOME NUMBERS OF CERTAIN SPECIES AND FORMS OF AGROPYRUM.] Sovet. Bot., No. 6, pp. 109–111. [In Russian. Summary in Plant Breed. Abs. 9 (1080): 310. 1939.]

(4) ARMSTRONG, J. M.
1936. HYBRIDIZATION OF TRITICUM AND AGROPYRON. I. CROSSING RESULTS AND DESCRIPTION OF THE FIRST GENERATION HYBRIDS. Canad. Jour. Res., Sect. C, Bot. Sci. 14: 190–202, illus.

(5) AVDULOW, N. P.
1931. KARYO-SYSTEMATISCHE UNTERSUCHUNG DER FAMILIE GRAMINEEN. Trudy Prikl. Bot., Genet. i Selek, Prilozh. (Bul. Appl. Bot., Genet. and Plant Breeding Suppl.) 44, 428 pp., illus. [In Russian. English summary, pp. 343–352.]

(6) CRASNIUK, A. A.
1935. [THE HYBRID SECALE CEREALE × AGROPYRUM CRISTATUM.] Sotsialist. Zern. Khoz. (Grain Prod. Jour.) No. 1, pp. [106]–114, illus. [In Russian. English summary, pp. 113–114. Also summary in Plant Breeding Abs. 5 (996): 323. 1935.]

(7) DERŽAVIN, A. I.
1936. [BREEDING PERENNIAL VARIETIES OF WHEAT AND OTHER PLANTS.] Selek. i Semen. 1936, No. 9: 20–24, illus. [In Russian. Summary in Plant Breeding Abs. 7 (946): 300–301. 1937.]

(8) DUKA, S. KH.
1938. [SELECTION METHODS FOR CULTIVATED PERENNIAL RYE.] *In* Symposium dedicated to the memory of V. N. Lubimenko, Ukrain. Akad. Nauk Inst. Bot., Kiev (Ukraine Acad. Sci., Inst. Bot.), pp. 409–413. [In Russian. Summary in Plant Breeding Abs. 9 (719): 202. 1939.]

(9) GUARD, A. T.
1938. STUDIES ON CYTOLOGY AND RESISTANCE TO LEAF RUST OF SOME INTERSPECIFIC AND INTERGENERIC HYBRIDS OF WHEAT. Amer. Jour. Bot. 25: 478–480, illus.

(10) JOHNSON, L. P. V.
1938. HYBRIDIZATION OF TRITICUM AND AGROPYRON. IV. FURTHER CROSSING RESULTS AND STUDIES ON THE F_1 HYBRIDS. Canad. Jour. Res., Sect. C, Bot. Sci. 16: 417–444, illus.

(11) ——— and MCLENNAN, H. A.
1939. AN ATTEMPT TO HYBRIDIZE ANNUAL AND PERENNIAL AVENA SPECIES. Canad. Jour. Res., Sect. C, Bot. Sci. 17: [35]–37, illus.

(12) KHIŽNJAK, N. A.
1936. [FORM DEVELOPMENT IN TRITICUM-AGROPYRUM HYBRIDS AND THE PRODUCTION OF PERENNIAL WHEATS.] Selek. i Semen. 1936, No. 12: 20–33, illus. [In Russian. Summary in Plant Breeding Abs. 7 (1212): 384–385. 1937.]

(13) ———
1936. [CYTOLOGICAL STUDY OF TRITICUM-AGROPYRUM HYBRIDS AND THE METHOD OF BREEDING PERENNIAL WHEATS.] Azovo-Chernomorskii Selek. Trudy 1: 25–30. [Summary in Plant Breeding Abs. 7 (951): 302–303. 1937.]

(14) ——— [KHIJNYAK, V. A.]
1938. [FORM-GENESIS IN WHEAT-AGROPYRUM HYBRIDS.] Acad. des. Sci. U. R. S. S., Bul., Ser. Biol., No. 3, pp. 597–626. [Summary in Plant Breeding Abs. (1023): 295–296. 1939.]

(15) KOSTOFF, DONTCHO.
1931–32. POLLEN ABORTION IN SPECIES HYBRIDS. Cytologia 3: 337–339, illus. [Summary in Plant Breeding Abs. 3 (403): 118. 1933.]

(16) ———
1937. INTERSPECIFIC HYBRIDS IN SECALE (RYE). I. SECALE CEREALE × SECALE ANCESTRALE, SECALE CEREALE × SECALE VAVILOVII, SECALE CEREALE × SECALE MONTANUM AND SECALE ANCESTRALE × SECALE VAVILOVII HYBRIDS. Acad. Sci. U. R. S. S., Compt. Rend. (Dok.) 14: [213–214], illus. [Summary in Plant Breeding Abs. 7 (1238): 392. 1937.]

(17) KUCKUCK, HERMANN.
1934. ARTKREUZUNGEN BEI GERSTE (VORLÄUFIGE MITTEILUNG). Züchter 6: 270–273, illus.

432645—41——4

(18) LJUBIMOVA, V. F.
1937. [THE PROBLEM OF TRITICUM-AGROPYRON HYBRIDS.] In Ogiz-Selkhozgiz, Ed. by N. V. Tzitzin, pp. 211–223. [Summary in Plant Breeding Abs. 9 (189): 47–50. 1939.]

(19) LONGLEY, A. E., AND SANDO, W. J.
1930. NUCLEAR DIVISIONS IN THE POLLEN MOTHER CELLS OF TRITICUM, AEGILOPS, AND SECALE AND THEIR HYBRIDS. Jour. Agr. Res. 40: 683–719, illus.

(20) MALLOCH, WALTER SCOTT.
1921. AN F_1 SPECIES CROSS BETWEEN HORDEUM VULGARE AND HORDEUM MURANIUM. Amer. Nat. 55: 281–286, illus.

(21) NEMLIENKO, N. E.
1939. [RESULTS OF WORK WITH TRITICUM-AGROPYRON HYBRIDS.] Selek. i Semen. 1939, No. 4: 16–19, illus. [Summary in Plant Breeding Abs. 9 (1442): 417. 1939.]

(22) NIELSEN, ETLAR L.
1939. GRASS STUDIES. III. ADDITIONAL SOMATIC CHROMOSOME COMPLEMENTS. Amer. Jour. Bot. 26: 366–372, illus.

(23) ——— AND HUMPHREY, L. M.
1937. GRASS STUDIES. I. CHROMOSOME NUMBERS IN CERTAIN MEMBERS OF THE TRIBES FESTUCEAE, HORDEAE, AVENEAE, AGROSTIDEAE, CHLORIDEAE, PHALARIDEAE, AND TRIPSACEAE. Amer. Jour. Bot. 24: 276–279, illus.

(24) PETO, F. H.
1930. CYTOLOGICAL STUDIES IN THE GENUS AGROPYRON. Canad. Jour. Res. 3: 428–448, illus.

(25) PODDUBNAJA-ARNOLDI, V.
1939. ON HYBRIDIZATION BETWEEN TRITICUM AND ELYMUS. Acad. des Sci. U. R. S. S. Compt. Rend. (Dok.) 24 (4): 378–381, illus. [Summary in Plant Breeding Abs. 10 (106): 24. 1940.]

(26) QUINCKE, F. L.
1940. INTERSPECIFIC AND INTERGENERIC CROSSES WITH HORDEUM. Canad. Jour. Res. Sect. C, Bot. Sci. 18: 372–373.

(27) RAW, A. R.
1939. INTERGENERIC HYBRIDIZATION. A PRELIMINARY NOTE OF INVESTIGATIONS ON THE USE OF COLCHICINE IN INDUCING FERTILITY. Victoria Dept. Agr. Jour. 37: 50–52, illus.

(28) REZNIČUK, S. P.
1938. [PERENNIAL RYE (PRELIMINARY COMMUNICATION)]. Sotsialist. Zern. Khoz. (Grain Prod. Jour.) No. 4, pp. 87–90, illus. [Summary in Plant Breeding Abs. 9 (1470): 425. 1939.]

(29) STEBBINS, G. L., JR., and LOVE, R. M.
1941. A CYTOLOGICAL STUDY OF CALIFORNIA FORAGE GRASSES. Amer. Jour. Bot. 28: 371–382, illus.

(30) TZITZIN, N. V.
1933. [THE TRITICUM × AGROPYRUM HYBRIDS.] Lenin. Acad. Agr. Sci. Siberian Inst. Grain Cult., Omsk, 1933, 101 pp. [Summary in Plant Breeding Abs. 5 (78): 24–25. 1934.]

(31) ———
1935. [MY EXPERIMENTS ON WHEAT.] Selek. i Semen. 1935, 3/11: 62–63. [In Russian. Summary in Plant Breeding Abs. 7 (593): 184. 1937.]

(32) ———
1936. [THE PROBLEM OF PERENNIAL WHEAT.] Selek. i Semen. 1936, No. 2: 21–27, illus. [In Russian. Summary in Plant Breeding Abs. 7 (593): 184. 1937.]

(33) ———
1936. [BREEDING TRITICUM-AGROPYRUM HYBRIDS.] Leningrad Akad. Selsk. Khoz. Nauk V. I. Bŭl. 1936, No. 10: 1–4. [In Russian. Summary in Plant Breeding Abs. 7 (1213): 385. 1937.]

(34) ———
1937. [WHAT DOES CROSSING WHEAT WITH AGROPYRUM GIVE?] Novoe v Sel'skom Khozjaistve (What is New in Agriculture), No. 7, 45 pp. [Summary in Plant Breeding Abs. 8 (1156): 350. 1938.]

(35) ———
1937. THE PROBLEM OF TRITICUM-AGROPYRUM HYBRIDS. Meaning Ogiz-Selkhozgiz, Ed. by N. V. Tzitzin. 235 pp. [Summary in Plant Breeding Abs. 9 (189): 47–50. 1939.]

(35–a) TZITZIN, N. V. [TSITSIN, N. V.]
1940. WHEAT AND COUCH GRASS HYBRIDS. Sci. and Cult. 6 (1): [18]–20.
(36) VAKAR, B. A. [WAKAR, B. A.]
1934. BASTARDE ZWISCHEN ARTEN DER GATTUNG TRITICUM UND ARTEN DER GATTUNG AGROPYRUM. Züchter 6: 211–215, illus.
(37) ———
1938. A CYTOLOGICAL STUDY OF F_1–F_6 TRITICUM VULGARE × AGROPYRUM INTERMEDIUM HYBRIDS. Acad. des Sci. U. R. S. S. Bul. Ser., Biol., No. 3, [627]–641, illus. [In Russian. English summary, p. 640. Also, summary in Plant Breeding Abs. 9 (1024): 296. 1939.]
(38) VERUSCHKINE, S. M.
1936. [THE PROSPECTS OF HYBRIDIZING WHEAT WITH AGROPYRUM.] Selek. i Semen. 1936, No. 2: 28–33, illus. [In Russian. Summary in Plant Breeding Abs. 7 (595): 184–185. 1937.]
(39) ——— [WERUSCHKIN, S. M.]
1936. [THE RELATIONSHIP BETWEEN THE GENERA AGROPYRUM AND TRITICUM.] Jour. Bot. de l'U. R. S. S. 21: 176–185, illus. [In Russian. Summary in German, pp. 184–185, and in English in Plant Breeding Abs. 9 (191): 50. 1939.]
(40) ——— [VERUSHKINE, S.] and SHECHURDINE, A.
1933. HYBRIDS BETWEEN WHEAT AND COUCH GRASS, FERTILE TRITICUM-AGROPYRUM HYBRIDS OF GREAT SCIENTIFIC AND PRACTICAL INTEREST. Jour. Hered. 24: 329–335, illus.
(41) VINALL, H. N., and HEIN, M. A.
1937. BREEDING MISCELLANEOUS GRASSES. U. S. Dept. Agr. Yearbook 1937; 1032–1102, illus.
(42) WHITE, W. J.
1940. INTERGENERIC CROSSES BETWEEN TRITICUM AND AGROPYRON. Sci. Agr. 21: 198–232, illus.

SOME ASPECTS OF THE PHYLLOTAXY OF TOBACCO[1]

By H. A. ALLARD

Senior physiologist, Division of Tobacco Investigations, Bureau of Plant Industry, United States Department of Agriculture

INTRODUCTION

The genus *Nicotiana*, which includes the true tobaccos, is characterized by an alternate disposition of the leaves throughout. An abnormal condition may sometimes bring about an occasional pair of opposite or whorled leaves in some varieties of tobacco, more especially in the rather anomalous short-day variety known as Maryland Mammoth, but this is too exceptional to be considered in any study of the phyllotaxy of tobacco.

HOW TO MAKE THE COUNT TO DETERMINE THE LEAF ARRANGEMENT

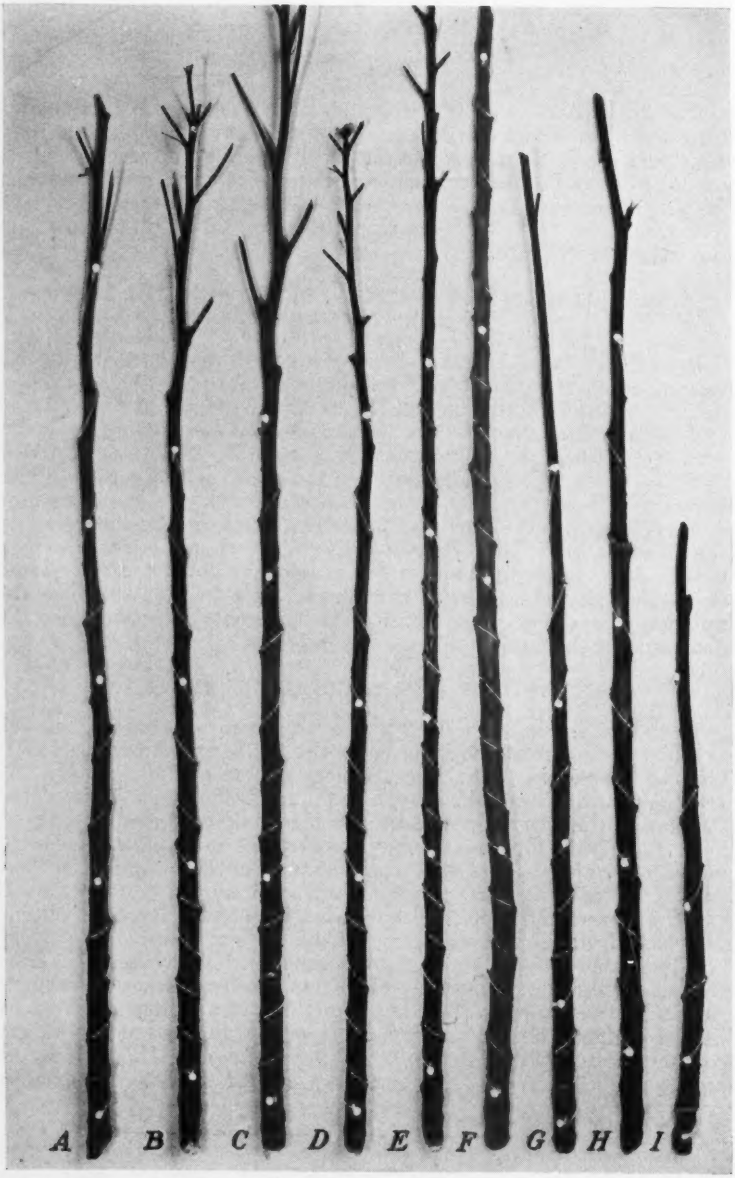
In order to determine the number of leaves present, counting from a certain leaf or node to the next leaf directly in line above the first one, the leaves counted must include the next younger leaf in proper order and all intervening leaves, but not the first leaf from which the count started. In this count it is very important to determine the true direction of the spiral, which may run to the left or to the right. This is found by passing to the nearest leaf above, which will be the next younger leaf from the starting point. The course is thus continued up the stem until the leaf directly above the first one is reached. In making this count, the number of encirclements of the stem is noted also. The phyllotaxy found is expressed as a fraction in which the numerator represents the number of encirclements of the stem and the denominator the number of leaves counted, as 2/5, 3/8, or 5/13.

CONSTANCY OF A PARTICULAR ARRANGEMENT

To determine the more fixed leaf arrangement in tobacco, it is well to study the leaf relations as far down the stalk as practicable. This is true for the reason that the morphological plan of the stem and leaf arrangement appears to be determined in the transition zone from root to shoot in the cotyledonary region of the embryonic plant. A count here, then, would more correctly express the inherent or primitive morphology of the plant than would a study of the secondary suckers or the top of the plant, which is farthest removed from the basal transition zone and more closely related to external forces governing flowering, fruiting, and senescence of the original plant.

While some tobacco plants show a constant leaf arrangement from the base to the top, others may change to a different fractional expression, usually of a lower order but sometimes of a higher order. As a matter of fact, tobacco shows a more or less inconstant phyllotaxy comparable to that of *Actinomeris alternifolia* (L.) DC., *Verbesina occidentalis* (L.) Walt., and many species of sunflower, including

[1] Received for publication August 14, 1941.


A
B
C
D
E
F
G
H
I

Helianthus tuberosus L. This behavior is not anomalous, but in reality is usual for many plants, and it would appear to be the normal expression for some strains of tobacco as well. These changes of leaf arrangement have been found in a number of commercial types.

While it may be true that many features of the plant are predetermined in the original embryonic axis, it would be absurd to conclude that every branch, sucker, or all the new shoots arising when the plant is cut to the ground are an exact unfolding of some preformed embryonic prototype simply expanded to natural size. Life is very plastic, and its primitive plans in many instances may be greatly modified by conditions of habitat.

DIRECTION OF THE SPIRAL

If one will take any tall stem, such as that of tobacco, having many successive nodes, or points of leaf insertion, fasten the end of a string to one node, as shown in figure 1, and follow up the stem in the proper course to the successively younger leaves from node to node, he will at once fix the left or right course of the spiral.

If the inclines of the string are to the right, the winding, as seen down the length of the stem, is counterclockwise; if the inclines are to the left, the winding is clockwise. These relations are shown in figure 1.

The direction of this spiral is one of the most fixed characters of the plant. While the actual leaf arrangement may change with respect to the number of leaves on the stem between points in line, the spiral course, probably originating somewhere in the transition zone between root and stem, is invariably maintained throughout in the normal plant and holds its primitive direction regardless of environmental influences. The writer has found no tobacco plant in any variety where this spiral course has reversed itself at any point, even in the suckers following topping or cutting back. Every variety shows both courses in different proportions and where selection for this character has not been followed the percentage of clockwise and counterclockwise nodes approaches a 50-percent ratio, which is one of normal chance where these two alternatives alone obtain. There is no reason, however, why strains could not be selected where ratios of one spiral pattern to the other would be very high, or even where either pattern, perhaps, might occur with a 100-percent certainty. When considered with other characteristics, this might serve, in some instances, to identify a certain strain.

FIGURE 1.—Different leaf arrangements found in some varieties of tobacco in the lower and upper parts of the main stem. The white string passes from the lowest leaf to the next younger leaf above, being fastened to each leaf node by a black tack, except to the nodes in a direct line above the first leaf, where large-headed roofing nails are used, the heads of which are painted white. *A–C*, Robinson Maryland Medium Broadleaf. *A*, 5/13 below, changing to 3/8 and 2/5 above; spiral course up the stem, counterclockwise. *B*, 5/13 below, changing to 3/8 above; spiral course, clockwise. *C*, 5/13 below, changing to 2/5 above; spiral course of leaves, counterclockwise. *D*, Rawling Medium Maryland Broadleaf, 5/13 below, 3/8 above; spiral course, clockwise. *E*, Maryland Mammoth, 5/13 below, changing to 3/8 above; spiral course, clockwise. *F*, Standup Maryland Mammoth, 5/13 throughout; spiral course, clockwise. *G*, Wilson Maryland Broadleaf, 3/8 below, changing to 2/5 above; spiral course, counterclockwise. *H*, Maryland Connecticut Broadleaf, 3/8 throughout; spiral course, clockwise. *I*, One Sucker, 2/5 throughout; spiral course, counterclockwise.

LEAF ARRANGEMENT OF SOME COMMERCIAL VARIETIES AND SPECIES

Table 1 shows the leaf arrangement and spiraling course of 43 Maryland Mammoth plants grown in the field at the Arlington Experiment Farm, Arlington, Va., in 1940. It will be seen here that tobacco plants not infrequently change their phyllotaxy in different portions of the stem.

TABLE 1.—*Phyllotaxy of 43 plants of Maryland Mammoth tobacco as noted from bottom to top of stem*

Phyllotaxy	Plants		Phyllotaxy	Plants	
Arrangement of leaves:	*Number*	*Percent*	Direction of spiral:	*Number*	*Percent*
5/13 throughout	12	27.6	Clockwise	20	46.5
3/8 throughout	8	18.6	Counterclockwise	23	53.5
5/13 below; 3/8 above	16	37.2			
3/8 below; 5/13 above	4	9.3			
5/13 below and above; 3/8 middle	2	4.6			
3/8 below and above; 5/13 middle	1	2.3			

Assuming that the arrangement of leaves on the lower part of the stem expresses the more primitive condition, this group of plants showed 69.4 percent with a 5/13 arrangement and 30.2 percent with a 3/8 arrangement.

In one plant of this group a few leaves were practically opposite or at the same node, and there seemed to be an accidental or anomalous phyllotaxy of 2/7 or 3/11. The clockwise and counterclockwise spiraling of the leaves, namely, 46.5 percent and 53.5 percent respectively, in this group of plants represented a nearly equal numerical occurrence.

In table 2 there is presented the phyllotaxy, or leaf arrangement and spiral course around the stem, of a number of commercial Ameri-

TABLE 2.—*Phyllotaxy of the leaves on the main stem below and suckers of some commercial American tobaccos and other varieties and species*

Variety or species	Total plants	Plants having indicated leaf arrangement						Spiral course				Phyllotaxy of suckers and related data
		2/5		3/8		5/13		Clockwise		Counterclockwise		
	No.	*No.*	*Pct.*	*No.*	*Pct.*	*No.*	*Pct.*	*No.*	*Pct.*	*No.*	*Pct.*	
Ambalema (South American)	6	0	0	6	100	0	0	1	16.6	5	83.3	A commercial variety in Argentina. No suckers present as a result of topping.
Catterton Maryland Broadleaf	24	0	0	24	100	0	0	11	45.8	13	54.1	May have been a selection from Johnson Root Rot Resistant × Maryland Connecticut Broadleaf. No suckers studied.
Little Dutch	8	0	0	8	100	0	0	5	62.5	3	37.5	All below showed a 3/8 phyllotaxy; 1 plant appeared to change to 2/5 above.
Little Orinoco	3	0	0	3	100	0	0	3	100	0	0	1 plant 3/8 below, but a tall sucker from topped portion above seemingly 2/5 below, 1/3 above, with spiral course clockwise throughout. 1 plant 3/8 to middle, 2/5 to top, and sucker from topped portion 3/8, and counterclockwise.

TABLE 2.—*Phyllotaxy of the leaves on the main stem below and suckers of some commercial American tobaccos and other varieties and species*—Continued

Variety or species	Total plants	Plants having indicated leaf arrangement						Spiral course				Phyllotaxy of suckers and related data
		2/5		3/8		5/13		Clockwise		Counterclockwise		
	No.	*No.*	*Pct.*	*No.*	*Pct.*	*No.*	*Pct.*	*No.*	*Pct.*	*No.*	*Pct.*	
Maryland Connecticut Broadleaf.	35	0	0	35	100	0	0	21	60	14	40	2 plants with top suckers 3/8 on all, and same spiral arrangement as on main stem.
Maryland Mammoth.	23	0	0	16	69.6	7	30.4	----	----	----	----	1 plant 3/8 below, 5/13 above on the main stem.
Maryland Standup Resistant.	20	0	0	20	100	0	0	9	45	11	55	Johnson Root Rot Resistant Burley × Maryland Connecticut Broadleaf. No data on suckers, as these had been removed.
Nicotiana glauca.	5	0	0	5	100	0	0	4	80	1	20	Young plants not yet in flower studied.
Nicotiana gossei (Australian).	24	20	83.3	4	16.7	0	0	[1] 6	33.3	12	66.6	Some untopped plants with 2 or 3 suckers, but the suckers showed same phyllotaxy as the main stem and the same spiraling throughout.
Nicotiana rustica Olson strain.	11	0	0	11	100	0	0	6	54.5	5	45.4	Plants mature in the field.
One Sucker	4	2	50	2	50	0	0	3	75	1	25	1 plant with 3/8 phyllotaxy, changed to 2/5 above; spiral arrangement clockwise throughout.
Rawling Fusarium Resistant.	34	0	0	29	85.3	5	14.7	18	52.9	16	47	1 plant with divided main stem below; both 3/8, with counterclockwise arrangement as in case of main stem on both branches. (Strain of Maryland Medium Broadleaf.
Robinson Maryland Medium Broadleaf.	44	4	9.1	33	75	7	15.9	21	60	14	40	1 plant with bifurcated stem 3/8 phyllotaxy and clockwise arrangement on both branches.
Robinson Maryland Medium Broadleaf (pure line).	32	0	0	13	40.6	19	59.4	----	----	----	----	1 plant with 3/8 phyllotaxy on main stem produced 2 suckers, each with 3/8 phyllotaxy; 1 plant with 5/13 phyllotaxy on main stem produced 2 suckers above, each with 3/8 phyllotaxy.
Samsun (Turkish).	4	0	0	4	100	0	0	4	100	0	0	All 4 plants with 3/8 phyllotaxy below and each with 1 sucker 2/5 above and clockwise throughout.
Standup Maryland Mammoth.	46	0	0	28	60.9	18	39.1	----	----	----	----	White Burley × Maryland Mammoth. No suckers present.
Wilson Maryland Broadleaf.	41	0	0	37	90.2		[4] 9.8	17	41.4	24	58.5	1 plant bifurcated below showed 3/8 phyllotaxy and counterclockwise arrangement on both branches.
Xanthi (Turkish).	4	4	100	0	0	0	0	2	50	2	50	1 plant 2/5 below on main stem, changing to 3/8 at top, clockwise throughout. 1 plant 2/5 below, 3/8 above, with a top sucker 1/3 phyllotaxy, clockwise on main stem and suckers. 1 plant 2/5 below, apparently 3/8 above, with 1 sucker above 2/5 and counterclockwise throughout.

[1] 18 plants observed as to spiral course.

can tobacco varieties, together with other varieties grown elsewhere commercially, and also data for the species *Nicotiana gossei* Domin., *N. glauca* Graham, and *N. rustica* L. The last-named is no longer grown in America for its leaf but is being tested for nicotine production because of its very high content of that alkaloid.

The data in table 2 are sufficient to indicate that the leaf arrangement on the lower part of the main stem of most of our commerical American tobaccos is expressed by the fractions 3/8 and 5/13. A 2/5 phyllotaxy is not usual, but has been found in a few plants of Robinson Maryland Medium Broadleaf; in One Sucker; in Xanthi, a Turkish variety; and in *Nicotiana gossei*, a little-known wild Australian species. The last-named species was characterized by a high 2/5 dominance, namely, 83.3 percent in a lot of 24 plants. There was no 5/13 occurrence in this species but 16.7 percent of the plants showed a 3/8 phyllotaxy.

Robinson Maryland Medium Broadleaf, represented by a progeny of 32 plants obtained from seed of 1 plant, gave the highest percentage of the 5/13 phyllotaxy, namely, 59.4 percent.

Both *Nicotiana glauca* and *N. rustica* had a leaf arrangement expressed by 3/8.

In most of these species and varieties of *Nicotiana*, the course of the spiraling on the stem was noted, and usually this was very variable. In one the counterclockwise arrangement was most frequent; in another the clockwise arrangement. Since there has been no conscious breeding or selection for this character, a condition of variability would naturally be expected.

In table 2, attention has been directed chiefly to the leaf arrangement on the lower part of the main stem, with the inference that the more primitive phyllotaxy would be more correctly represented there. Many tobacco plants change their phyllotaxy, however, and it would seem that the change is usually to a lower order of the primary series rather than to a higher order. Suckers may or may not continue the same fractional arrangement found on the main stem. A 1/3 fractional arrangement has been found on some suckers, but this has never been observed on the main stem of any tobacco, although conceivably it may occur.

SUMMARY AND CONCLUSIONS

A general survey of phyllotaxy in tobacco is presented.

There are several normal leaf arrangements in varieties and species of *Nicotiana*, those on the main stem being expressed by the fractions 2/5, 3/8, and 5/13, where the numerator indicates the number of encirclements around the stem and the denominator the number of leaves encountered beginning with a given leaf and passing upward to successively younger leaves around the stem until a leaf directly in line with the first is reached. The count begins with the next leaf above the starting point and continues to and includes the next leaf alined with the one at the starting point.

Tobacco may present a 1/3, 2/5, 3/8, or 5/13 arrangement of the leaves. The 1/3 arrangement has been found only in the suckers of a very few plants of those normally showing the low fraction order 2/5, although severe cutting back or maltreatment that produces abnormal growth and branching may be expected to produce 1/3,

2/5, or 3/8 arrangements in any plant where the main stem shows a more complex phyllotaxy.

The 2/5 phyllotaxy is not usual in the large-leaved American varieties but appears to be the prevalent form in some small-leaved Turkish varieties, such as Xanthi.

A 3/8 and a 5/13 phyllotaxy are the common arrangements of the leaves in tobacco, these occurring in varying proportions. There is no reason, however, why breeding and rigid selection should not establish a high frequency of any phyllotaxy normal to the tobacco plant. It is possible that the phyllotaxy expressed by the fraction 5/13 represents a higher or more recent development in tobacco, more especially since a lower or simpler order may be enforced by changes in the age of the stem or branches, or by mutilations such as cutting down or back, by this means enforcing rejuvenation of growth and expressions farther removed from the original and more primitive phyllotaxy nearest the earlier embryonic tissues of the main stem.

As in many other plants, the phyllotaxy of tobacco is more or less variable, owing to internal or external factors. The more constant or primitive arrangement is most likely to occur on the lower part of the main stem rather than near the inflorescence or on suckers following cutting or topping.

The spiral arrangement upward to successively younger leaves may express itself as a clockwise or a counterclockwise course. This is a much more rigidly enforced relationship in the constitution of the plant than the fractional phyllotaxy encountered. While the fractional expressions may change on different parts of the stem or in the branches or suckers, the spiral course once established remains fixed to a very high degree. It probably never reverses itself except with very abnormal torsions, and this has not been observed by the writer.

RELATION OF ASCORBIC ACID CONCENTRATION IN JUICE OF FLORIDA GRAPEFRUIT TO VARIETY, ROOTSTOCK, AND POSITION OF FRUIT ON THE TREE [1]

By PAUL L. HARDING, *horticulturist*, and EDWARD E. THOMAS, formerly *minor scientific helper, Division of Fruit and Vegetable Crops and Diseases, Bureau of Plant Industry, United States Department of Agriculture* [2]

INTRODUCTION

During recent years much interest has been shown in the nutritive values of different foods in both human and animal diets. In part, at least, this increasing interest has resulted from the chemical identification of some of the common vitamins and the development of accurate and rapid methods for determining the vitamin content of various foods and food products.

The seasonal changes in the ascorbic acid content of the juice of Florida oranges have been reported by Harding, Winston, and Fisher.[3] [4] These authors pointed out that no correlation was found between the ascorbic acid content of orange juice and its quality as judged by taste. They also reported a significant difference in ascorbic acid concentration between fruit exposed to the sun and shaded, fruit, the exposed fruit having the higher ascorbic acid concentration.

The investigation reported herein had the following objectives: (1) To determine the ascorbic acid content of the juice of Florida grapefruit (*Citrus grandis* (L.) Osbeck), and (2) to determine whether the ascorbic acid content is influenced by rootstocks, variety, and the position of the fruit on the tree (outside or exposed fruit compared with inside or shaded fruit).

MATERIAL AND METHODS

The grapefruits used in this study were collected at random from the "regular bloom" of mature trees located in a commercial grove on Merritt Island, Fla. (east coast). The trees had slightly sparse but normal foliation typical of the section. The fruits were from three different plots of five trees each: Marsh grapefruit grown on rough lemon rootstock, Marsh grapefruit grown on sour orange rootstock, and Foster (pink) grapefruit grown on sour orange rootstock.

---

[1] Received for publication June 9, 1941.

[2] Acknowledgments are due D. F. Fisher, principal horticulturist, Division of Fruit and Vegetable Crops and Diseases, for suggestions regarding this investigation; J. K. Holloway, associate entomologist, Bureau of Entomology and Plant Quarantine, for assistance in planning the work and analyzing the data; and M. H. Haller, associate pomologist, Division of Fruit and Vegetable Crops and Diseases, for assistance in the statistical analysis of the data.

[3] HARDING, PAUL L., WINSTON, J. R., and FISHER, D. F. SEASONAL CHANGES IN THE ASCORBIC ACID CONTENT OF JUICE OF FLORIDA ORANGES. Amer. Hort. Sci. Proc. (1938) 36: 358–370. 1939.

[4] ——— WINSTON, J. R., and FISHER, D. F. SEASONAL CHANGES IN FLORIDA ORANGES. U. S. Dept. Agr. Tech. Bul. 753, 89 pp., illus. 1940.

Twenty-six fruits were picked from each of 15 trees: 13 fruits from outside branches well exposed to sunlight, and 13 from inside partially shaded branches. They were picked on November 10, 1939, and placed in storage at 32° F. at the United States Department of Agriculture Horticultural Field Laboratory, Orlando, Fla., and were analyzed within a few days.

The ascorbic acid or vitamin C content of the juice was determined individually for each of the 13 outside and of the 13 inside fruits from each of the 5 trees in the 3 plots, or for 390 fruits. In making the determinations, the juice was extracted by hand squeezing and then strained to remove pulp and seeds. Determinations were made by the method described by Bessey and King,[5] which consists essentially of the titration of grapefruit juice with a solution of freshly prepared sodium 2,6-dichlorobenzenoneindophenol (2,6-dichlorophenolindophenol) which had been standardized against fresh commercial crystalline ascorbic acid.

RESULTS

In table 1 are given the ascorbic acid concentration (milligrams per milliliter of juice) in 390 individual fruits, the total in the samples from the 13 fruits from each exposure on each tree, and the average amount in a milliliter of juice. Totals and means calculated from the individual fruit determinations in table 1 are presented in table 2. The data are analyzed statistically [6] [7] [8] in tables 3 and 4.

The determinations indicate that Florida grapefruit is a rich source of ascorbic acid. The values were in close agreement with unpublished data from other producing districts in the State. On a milligram per milliliter of juice basis, the values reported for grapefruit in table 1 are slightly lower than those that have been reported for Florida oranges [9] for comparable dates. However, since grapefruit is generally much larger than oranges and is usually served in halves, it is quite possible that the total ascorbic acid consumed by one person at a meal would be similar to that in an ordinary serving of orange juice.

[5] BESSEY, OTTO A., and KING, C. G. THE DISTRIBUTION OF VITAMIN C IN PLANT AND ANIMAL TISSUES AND ITS DETERMINATION. Jour. Biol. Chem. 103: 687-698. 1933.

[6] BARTLETT, M. S., and GREENHILL, A. W. THE RELATIVE IMPORTANCE OF PLOT VARIATION AND OF FIELD AND LABORATORY SAMPLING ERRORS IN SMALL PLOT PASTURE PRODUCTIVITY EXPERIMENTS. Jour. Agr. Sci. [England] 26: [258]-262. 1936.

[7] FISHER, R. A. THE DESIGN OF EXPERIMENTS. 252 pp. Edinburgh and London. 1935.

[8] SNEDECOR, GEORGE W. STATISTICAL METHODS APPLIED TO EXPERIMENTS IN AGRICULTURE AND BIOLOGY. Ed. 3, 422 pp. Ames, Iowa. 1940.

[9] See footnote 3, p. 57.

TABLE 1.—*Ascorbic acid content of the juice of fruits produced on outside and inside branches of Marsh and Foster grapefruit at Merritt Island, Fla., Nov. 10, 1939*

Variety and rootstock	Ascorbic acid per milliliter of juice of individual fruits from—									
	Outside branches of tree No. —					Inside branches of tree No. —				
	1	2	3	4	5	1	2	3	4	5
	Mg.	*Mg.*	*Mg.*	*Mg.*	*Mg.*	*Mg.*	*Mg.*	*Mg.*	*Mg.*	*Mg.*
Marsh on rough lemon rootstock	0.41	0.40	0.53	0.48	0.47	0.41	0.41	0.48	0.49	0.48
	.40	.40	.50	.47	.46	.40	.39	.47	.48	.47
	.40	.40	.50	.47	.46	.38	.39	.47	.47	.46
	.40	.39	.49	.47	.44	.37	.37	.46	.47	.45
	.39	.38	.47	.46	.44	.36	.37	.45	.45	.45
	.39	.38	.47	.45	.44	.36	.37	.45	.45	.44
	.39	.38	.46	.45	.44	.35	.36	.44	.44	.44
	.39	.37	.46	.45	.44	.35	.36	.44	.44	.43
	.37	.37	.46	.45	.43	.35	.36	.44	.42	.43
	.37	.37	.46	.44	.43	.35	.34	.44	.42	.43
	.37	.36	.46	.44	.43	.34	.33	.44	.42	.43
	.34	.35	.45	.44	.43	.34	.33	.42	.40	.41
	.33	.35	.44	.43	.41	.33	.32	.41	.40	.40
Total	4.95	4.90	6.15	5.90	5.72	4.69	4.70	5.81	5.75	5.72
Average	.38	.38	.47	.45	.44	.36	.36	.45	.44	.44
Marsh on sour orange rootstock	.47	.44	.41	.42	.43	.43	.39	.41	.41	.40
	.44	.44	.41	.41	.42	.41	.38	.39	.38	.40
	.44	.43	.39	.41	.42	.40	.37	.38	.37	.39
	.44	.41	.39	.41	.41	.40	.37	.38	.37	.38
	.44	.41	.38	.40	.41	.39	.37	.38	.37	.38
	.44	.41	.38	.40	.41	.39	.36	.37	.36	.38
	.43	.40	.38	.40	.40	.39	.36	.36	.36	.37
	.42	.39	.38	.40	.40	.37	.34	.36	.35	.37
	.42	.39	.38	.39	.40	.37	.33	.36	.35	.37
	.42	.39	.37	.39	.39	.37	.33	.35	.35	.36
	.41	.39	.37	.39	.37	.37	.32	.35	.35	.36
	.39	.38	.37	.38	.36	.36	.32	.34	.34	.35
	.39	.37	.36	.38	.35	.35	.32	.34	.34	.35
Total	5.55	5.25	4.97	5.18	5.17	5.00	4.56	4.77	4.70	4.86
Average	.43	.40	.38	.40	.40	.38	.35	.37	.36	.37
Foster (pink) on sour orange rootstock	.46	.46	.44	.46	.48	.42	.45	.47	.46	.43
	.44	.46	.44	.46	.47	.41	.43	.44	.44	.43
	.44	.44	.43	.46	.46	.41	.42	.43	.44	.42
	.44	.44	.43	.45	.44	.41	.42	.41	.44	.42
	.44	.43	.42	.44	.44	.39	.41	.41	.42	.41
	.43	.43	.42	.43	.43	.39	.41	.41	.42	.41
	.41	.43	.42	.42	.43	.38	.40	.40	.41	.41
	.40	.42	.41	.41	.41	.38	.40	.39	.41	.40
	.40	.39	.41	.41	.41	.38	.40	.39	.40	.40
	.39	.39	.40	.41	.41	.37	.39	.39	.39	.40
	.39	.38	.40	.41	.39	.37	.36	.38	.39	.40
	.39	.37	.40	.40	.37	.36	.36	.38	.37	.39
	.38	.34	.39	.39	.36	.33	.34	.38	.36	.37
Total	5.41	5.38	5.41	5.55	5.50	5.00	5.19	5.28	5.35	5.29
Average	.42	.41	.42	.43	.42	.38	.40	.41	.41	.41

TABLE 2.—*Total and mean ascorbic acid determinations calculated from individual fruit determinations listed in table 1*

Variety and rootstock	Total (per 65 ml. of juice) from fruit from indicated position		Total (per 130 ml. of juice)	Mean (per 13 ml. of juice)
	Outside	Inside		
	Milligram	*Milligram*	*Milligram*	*Milligram*
Marsh on rough lemon rootstock	27.62	26.67	54.29	5.43
Marsh on sour orange rootstock	26.12	23.89	50.01	5.00
Foster on sour orange rootstock	27.25	26.11	53.36	5.34
Total (per 195 ml. of juice)	80.99	76.67		
Total (per 390 ml. of juice)			157.66	
Mean (per 13 ml. of juice)	5.40	5.11		5.26

TABLE 3.—*Analysis of variance of the ascorbic acid determinations listed in table 1*

Variance due to—	Degrees of freedom	Sum of squares	Mean square	Variance ratio [1]
Sources	2	0.0780	0.0390	2.10
Within sources (between trees within sources)	12	.2236	.0186	
Total whole trees	14	.3016	.0215	
Positions	1	.0479	.0479	59.88**
Position×source	2	.0073	.0037	4.62*
Position×tree within sources	12	.0100	.0008	
Total half trees	29	.3668	.0126	
Within half trees	360	.2010	.0006	
Grand total	389	.5678		

[1] **=Highly significant, P=0.01 (odds 99:1). *=Significant, P=0.05 (odds 19:1).

TABLE 4.—*Analysis of the variance of the ascorbic acid concentration of fruits of Marsh grapefruit grown on rough lemon and sour orange rootstocks and of those of Foster and Marsh grapefruit grown on sour orange rootstock*

MARSH GRAPEFRUIT ON ROUGH LEMON AND SOUR ORANGE ROOTSTOCK

Variance due to—	Degrees of freedom	Sum of squares	Mean square	Variance ratio [1]
Between sources	1	0.9159	0.9159	2.59
Trees within sources	8	2.8340	.3543	
Positions	1	.5056	.5056	37.45**
Position×source	1	.0820	.0820	
Position×trees within sources	8	.1078	.0135	
Total	19	4.4453		

FOSTER AND MARSH GRAPEFRUIT ON SOUR ORANGE ROOTSTOCK

Between sources	1	0.5612	0.5612	15.85**
Trees within sources	8	.2834	.0354	
Positions	1	.5679	.5679	46.55**
Position×source	1	.0593	.0593	
Position×trees within sources	8	.0979	.0122	
Total	19	1.5697		

[1] **=Highly significant; P=0.01 (odds 99:1).

The analysis of variance of the ascorbic acid determinations (tables 3 and 4) shows a highly significant difference between the two position means (determinations on juice from fruit produced on outside and inside branches) and (table 4) between Foster and Marsh grapefruits on sour orange rootstock (determinations on juice from fruits produced on different varieties). The ascorbic acid concentration in fruit produced on the inside branches was lower than that of fruit produced on the outside branches.

There was a significant interaction between position and source. The differences between positions were not proportional in the three sources. This, however, is of minor importance in view of the highly significant difference due to positions. The results are of particular interest because the trees were sparsely foliated and the inside fruit was but partly shaded.

In table 4 the analysis of variance was made to determine the difference in ascorbic acid concentration in Marsh grapefruit grown on rough lemon and sour orange rootstocks. The ascorbic acid concentration was slightly higher in the fruit grown on rough lemon. These differences, however, were not significant.

Table 4 presents also an analysis of variance of determinations made on the juice of Foster and Marsh grapefruit both grown on sour orange rootstock. These data indicate a highly significant difference ($P=0.01$) between the two varieties; the Foster variety had the higher ascorbic acid concentration.

SUMMARY

The ascorbic acid concentration was determined for 390 individual grapefruits picked from outside and inside branches of 5 trees each of Marsh grapefruit grown on rough lemon rootstock, of Marsh grapefruit grown on sour orange rootstock, and of Foster (pink) grapefruit grown on sour orange rootstock.

The results showed that Florida grapefruit from various sources has high antiscorbutic properties. In this experiment the average ascorbic acid concentration was 0.40 milligram per milliliter of grapefruit juice.

A relatively small but consistent difference was found in the ascorbic acid concentration in fruits obtained from outside and inside branches from the three sources; the outside fruit had the higher ascorbic acid concentration. This difference was found to be significant at the 1-percent level.

The ascorbic acid concentration in fruits from the Marsh variety grown on rough lemon and on sour orange rootstocks was determined. A statistical study of these data showed a higher ascorbic acid content per milliliter of juice of fruit grown on rough lemon rootstock, but the difference was not significant.

The ascorbic acid content of juice of fruits on the Marsh and Foster (pink) varieties of grapefruit, both of which were grown on sour orange rootstock, was also determined. The analysis of variance of these data indicates a higher ascorbic acid concentration in the Foster fruit. This difference was found to be highly significant ($P=0.01$).

432645—41——5

U. S. GOVERNMENT PRINTING OFFICE: 1941